HAZARDS ANALYSIS, CRITICAL CONTROL POINTS IN THE PREPARATION OF AN ENRICHED CEREAL-BASED BEVERAGE (Soy-*Kununzaki*)

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August, 2006

DECLARATION

I here by declare that this thesis titled **'Hazards Analysis, Critical Control Points in the preparation of an enriched cereal-based beverage (Soy-*Kununzaki)*'** was written by me and it is a record of my own research work. It has not been presented before in any previous application for a higher degree. References made to publish and unpublished literatures have been duly acknowledged.

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Certification

This thesis titled: '**Hazards Analysis, Critical Control Points in the Preparation of an enriched cereal-based Beverage (Soy-*kununzaki*)**' by **Danbaba Nahemiah** (M.Tech/SSSE/930/03/04) meets the regulations governing the award of the degree of Master of Technology (**M.Tech. Microbiology**) of the Federal University of Technology, Minna, and is approved for its contribution to scientific knowledge and literary presentation.

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To my wife Cpl (Mrs.) Theresa Danbaba

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Abstract

The hazard analysis critical control point (HACCP) procedures were adopted to identify hazards and critical control points associated with fortified cereal based beverage-Soy-*kununzaki* produced by five randomly selected producers. Materials for were obtained from the national Cereals Research Institute, Badeggi and modern market Bida, Niger State. Physical, chemical and microbiological attributes of the raw materials and product during fermentation were evaluated using standard methods. Physical hazards identified include stone, broken glass pieces, other plant seeds, straws, insects, plastics, and microbial contamination. The mean total bacterial counts on the raw material used were $4.18x10^4$ CFU to less than $1.00x10^1$CFU. Coliform count ranged from $5.36x10^4$CFU to less than $1.00x10^1$CFU. While mold and yeast counts ranged from $6.20x10^3$CFU to $2.21x10^3$CFU. A statistical analysis using Completely randomized design analysis of variance showed that there is a significant increase in the bacterial count as well as the yeast and mold counts. Organisms isolated include *Bacillus, Staphylococcus, Micrococcus, Klebsiela, Shegella* and *Lactobacillus* for bacteria and *Aspergillus, Mucor, Rhizopus nigrican, Penicillium* and *Trichophyton* for molds from raw materials. During the processing, *Trichosporon, Torulopsis, Saccharomyces, Candida,* and *Cryptococcus* were the species of yeast identified. While *Bacillus, Lactobacillus, Klebsiela, Corynebacterium, Staphylococcus, Listeria* and *Micrococcus* were the bacteria identified. Hence there are high risks associated with the production and consumption of Soy-*kununzaki* if good manufacturing practice is not properly adhered to.

Chapter One

1.0.0 Introduction

Food manufacturers and regulatory agencies relied heavily on microbiological testing of finished products as a means of determining their safety. This practice has been on for many years. Several factors has forced the re-evaluation of this traditional approach, these are consumers demand for more natural foods or food for which the traditional safety guards against microbiological hazards are less evident, the emergence of strains of pathogenic microorganisms for which traditional control protocol are inadequate, and, great increase in the trade of food commodities among different countries whose concern, standards and approach to food safety and quality assurance process differ from ours, have all put stresses on the traditional food preparation and quality assurance approaches. The hazard analysis critical control point (HACCP) is recognized as more effective than the conventional end-product sampling practices, because it places more emphases on prevention and control of potential hazards (FAO, 2004). The HACCP approach to food safety management is recommended by the International Commission on Microbiological Specification for Foods (ICMSF, 1986), the National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 1992), and the Codex Alimentarius Commission (CODEX, 1993) and is being instituted through

out the world (CFP, 2004). The HACCP is based on seven key principles: the potential hazards in foods are identified and their risks assessed; critical control point (CCP), the point in the process where control could be exercised to minimize or eliminates the potential hazards, are identified; specifications are established for each CCP; corrective action to be taken when deviation occur at the CCP are identified; a record is kept of the process flow chat; and verification procedures are put in place to ensure that the HACCP is functional are established. Thus, HACCP identifies hazards and preventive measures for their control. Its effect is to focus control at the CCPs, and to provide a bases on which the performance of food production system and the expected safety of the food processed by it can be assessed immediately by the processes (Stevenson, 1990; Price, et al., 1993). According to these principles, a risk assessment will take into consideration: the evidence of hazard to health; the microbiology of the food; the likelyhood and consequences of microbiological contamination and/or growth during subsequent handling, storage and use; the category of consumers at risk; and, cost-benefit rational associated with the application of the specification (CFP, 2004).

Kunu is a generic name used in describing all kinds of non-alcoholic beverages (cereal based) with specifications usually attached to denote the based cereal grain. *Kununzaki* therefore, refers to sweet *kunu,* which is a traditional nonalcoholic beverage consumed widely in the northern part of Nigeria (Nkama, 1993;

Anounye, 1997). It is usually spiced with ginger, cloves, red pepper, black pepper, and cinnamon collectively known as *kayan yaji* in Hausa. Its production is traditionally carried out on small-scale batches, being made widely and served as beverage for visitors or sold by street vendors as beverage. The methods of production produce a product with characteristic sour taste. The taste is as a result of fermentation by microorganisms of the mash, producing metabolites including acids. These acids contribute to the development of aroma and flavor in the beverage (Faparusi, 1970; Anounye, 1997).

To improve the nutritive value of the product, legumes are added as a means of increasing its protein content. A great deal of fruitful research is being done on plants as cheap source of protein, minerals and vitamins in most parts of the World. Legumes are such group (El-Tinay, et al., 1985). The usefulness of legume in the production of high protein foods in meeting the needs of the vulnerable group of the population is now well recognized, and several high protein foods have been developed industrially in different parts of the World. The high costs of these industrially developed high protein foods makes them out of reach for the low and average income persons in Africa (Nkama, 1993). Alternatives for the processing of industrially produced high protein foods that will meet the needs of all in Africa seems to be the increased use of improved traditional food preparation techniques (Nkama, 1993; Sanni, 1997). An improved technique for the production

of a cereal-based beverage enriched with soybean has been developed at the National Cereals Research Institute (NCRI), Badeggi, and similar work has been carried out at the Federal Institute of Industrial Research (FIIRO), Oshodi, Lagos. The new technology involves the addition to the cereal base soybean for the production of *kununzaki* (Anounye, 1997; Bankole and Olatunji, 2001).

Soy-*kununzaki* is distributed with minimal packaging and has a limited shelf life of about one day at ambient temperature (Efiuvwevwere and Akoma, 1997).

Improvement of the traditional technique involves quality and process optimization, which requires critical research into the various factors affecting the spoilage and safety of the food to the consumer (Gilbert, et al., 2000), particularly a ready-to-eat food (Jideani, et al., 2001) like soy-*kununzaki*. A tool with which food industries all over the world are adapting to aid in the production of safe and wholesome foods is the HACCP system (Sanni, 1997; Solomon, 2001 and McDonough, 2002). The system is designed to prevent the occurrence of potential food safety hazards (USDA, 2002). Street vended foods have shown epidemiological links with several illness and laboratory results have shown high counts and the presence of food borne pathogens (Bryan, et al., 1992 and Yasmean, 2001). The perceived food safety hazards associated with steps, points or procedure in soy-*kununzaki* production process needed to be identified and documented for effective quality assurance protocol. This would include

microbiological hazards, physical and chemical hazards. Since soy-*kununzaki* is a high water activity (aw) product (the amount water available for bacterial growth), nutrients, and pH value, the product is suitable for microbial growth. Therefore, the monitoring and control of these organisms is of critical need. For this purpose, soy-*kununzaki* producer washes soybeans and other ingredients thoroughly with water, which decreases the number of microorganisms on/in the materials, and protects washed raw materials from recontamination during processing (Murai, 1997). But it is a fact that some soybeans microorganisms such as *Bacillus* species have heat resistant spores. Rinsing them even thoroughly with hot water cannot eliminate these spores (Kijima,et al., 1997). Electrolyzed water produced by electrolysis of common salt (NaCl) has been tested for the control of microorganisms on raw materials during food production (Zhaohui, et al., 2002). These water has been reportedly been shown to be effective in killing a range of food poisoning bacteria, including *E. coli, Salmonella* and *Listeria* and does not affect the taste and appearance of the food product (HNSI, 2000).

1.1.0 Objective of the study

The objective of the study is therefore, to conduct hazard analyses for soy-*kununzaki* preparation, identification of critical control points (CCPs), develop production flow chat showing the critical control points, identification and characterization of microorganisms at the critical control point levels, evaluation of

nutritive value of soy-*kununzaki* and test of the potential of electrolyzed water in the control of microorganisms in soy-*kununzaki* processing.

1.2.0 Justification of the study

Kununzaki is popular among the people of the northern part of Nigeria. Women earn considerable amount of income in selling this product, this has improve their poverty level, therefore, process and product optimization will improve the quality of this product and this implies more patronage and income, which is the cardinal point of the government poverty eradication program, the National Economic Empowerment Development Strategies (NEEDS) and this comes under the Millennium Development Goal Challenges in Nigeria. *Kununzaki* has been popular because of its characteristic sour-sweet taste and milky-creamy appearance, and its low viscosity and cost compared with the readily available carbonated beverages, which are beyond the reach of an average Nigeria. It is of this importance that it can hardly be missed in social gatherings among the several consuming communities. Since consumption and popularity are high, the nutritive and safety characteristics of this product should be of up most priority with regard to its regulation. The quality and safety issues are important so that markets are not compromised by the sale and consumption of low quality, unsafe and substandard foods. For the safety of human foods, food borne pathogens constitutes the greatest hazards. The hazard analysis critical control point (HACCP) program is becoming

an international requirement for the development and registration of foods. The Codex Alimentarius Commission, the international food standard-setting body overseen by the United Nation (UN) agencies, the Food and Agricultural Organization (FAO), and World Health Organization (WHO), now recommended HACCP adoption all over the World (FAO, 1998; CFP, 2002). Food industries are rushing to implement the process to insure the safety of their product and meet the stringent quality assurance requirement of the international and local standard organizations to survive the competitive World of food market (FAO, 1998). The use of electrolyzed water in food industries for the control of microorganisms in foods all over the world is due to its tested effectiveness, low cost of production, adaptability, environmental friendliness and ease of obtaining is receiving greater attention and we can not afford to miss these quality assurance technology. Hence the need to conduct research into a cheap, adaptable and sustainable quality management protocol and microbial control technique for the production of a widely consumed ready-to-eat food like soy-*kununzaki*.

Chapter Two

2.0.0 Literature Review

Nuru (1985) advocated the need for the utilization of legumes in the development of high protein foods that will meet the average daily protein, minerals and vitamins intake per person in the developing countries of Africa, including Nigeria, which is below the recommended daily requirements of 85g per day by the British Medical Association. The traditional methods of preparing high protein foods from legumes provide an obvious starting point in the research for improved methods for cereal-legume utilization (Nkama, 1993). The traditionally processed foods product of cereal-legume combination comprises a great diversity of products, all prepared by a variety of processes (Nkama, 1993; NFPA, 1993). Some of the widely known legume-cereal based products are *kununshinkafa, kunungida, kunun tsir, Ndakuwa* etc majority of these products including soy-*kununzaki* are sold as street foods. The safety of these products is critical to the food regulatory agencies, processors and consumers a like (NFPA, 1993; Sanni, 1997; Solomon, 2001).

2.1.0 Raw materials used for the production of Soy-*kununzaki*

2.1.1 Soybeans

Soybeans (*Glycine max (L) merr*) occupy a premier position as world crop because of their high and virtually unrivalled, protein content and also because they are rich sources of edible vegetable oil. They are legumes and commercial varieties are usually spherical and yellow, although black, brown and green soybeans exist. They are about 8% hull, 90% cotyledon and 2% hypocoyl (McDonal, 1985). Soybean proteins are rich in lysine and threonine, but poor in methionine and tryptophan. The high level of lysine makes food legumes an ideal supplement for cereals, which is deficient in this amino acid. Feeding trials in Central America with rats has shown that a mixture of 90% cereal and 10% soybeans not only improves the quality of the protein in the diet but also increases the gain in body weight (McDonal, 1985; FAO, 1977). Though, legumes are known to contain toxic substances, including cyanogenic glycosides, trypsin inhibitors, hemaglutinins, lathyric factors and farism factors. Most of these factors are inhibited by cooking (FAO, 1977). Aflatoxins are not considered a problem in soybeans storage, because Hesseltine, et al., (1966) reported that flatoxins were not detected on soybeans inoculated with Aspergillus *flavus,* although, aflatoxins was detected in sterilized soybeans inoculated with *A. parasiticus.* Fungi known to damage

soybean include *Diaporthe phaseolorum, Collectotrichum dematium* var. *truncate* and *cercospor kikuchii* (McDonal, 1985). The use of soybean for beverage formulation has been reported by several authors (Karl, 1987; Iwe and Anounye, 1990; Kieko, et al., 1992; Anounye, 1997, Bankole and Olatunji, 2001 and Akoma, et al., 2002). All these authors are of the opinion that good high protein food drink and other food formulation could be obtained from soybean to meet the daily protein and energy requirement of the individuals. The increasing utilization of soybeans in the processing of foods has often been limited by the rapid enzyme reactions in cracked soybeans, which according to Nilson, et al., (1979) and Karl, (1987) lead to the development of off flavor in soybean products. There is also the natural long cooking time associated with soybeans (Karl, et al., 1987), which generally hampers their utilization especially at the home level. Added to this is the short shelf-life of soybeans products and the flatulence effects which are due to the presence of low molecular weight carbohydrate (Rackis, 1979).

In Nigeria, Nestle Foods Plc started local manufacturing and distribution of soybeans-maize cereal food for infants, and a new breakfast food has recently been put on the market. At Kursey children homes in Ogbomoso, Western Nigeria, the staff feed soybean-based foods to sick and malnourished children. Although the soybean makes up only a part of the diet, they supply most of the protein. Another limitation to the utilization of soybeans in beverage and food formulation is the

characteristic beans flavor. In the orient, people appreciate the beany flavor, but consumers in Africa, Europe and North America find it objectionable. The flavor is caused by a chemical reaction initiated by iso-enzymes of lipoxygenase contained in the beans. The iso-enzymes oxygenate polyunsaturated fatty acids containing cis, cis-1, 4-pentadiene double bonds located between carbon 6 and 10 from the methyl end of the fatty acid. In combination with linoleic acid, the iso-enzymes produce hydroperoxides that are responsible for the beany flavor. For this reaction to occur, lipoxygenase needed linoleic acid, water and disruption of the cell containing the substrate. The soaking of intact soybeans will not cause the reaction but adding water and ground or milling of soybeans will. Lipoxygenase can easily be destroyed by heat

2.1.2 Peal Millet

Millet (*Pennisetum typoids*) is a staple food crop for large segment of population in Africa and Asian countries where it contributes a major part of dietary nutrients. Peal millet is consumed in various forms depending on taste, cultural and food habits of people. Besides providing protein and calories peal millet is a good source of minerals such as calcium, iron, zinc, copper and manganese (Sunita, et al., 1988). Peal millet has high content of methionine and tryptophan but poor in lysine, this makes supplementation of the cereal with soybeans advantageous (FAO, 1977).

2.1.3 Spicies

In northern Nigeria, where *kununzaki* is mainly consumed, spices mainly ginger (*Zingiber officinale*), black pepper (*pipper guinease*), cloves (*Euginia caryophllata*) and cinnamon (*Xylopia aethiopica*) collectively called kayanyaji in Hausa language is added to the product (Anounye, 1997). The amount added depends on the individual preference. Adeyemi and Umar (1994) reported a combination of 65% ginger, 5% cloves, 25% red pepper, and 5% black pepper and were preferred by a panel of judges with about 20% clove. Onruarah, et al., (1987) had reported the adding of spices to *kununzaki* in the ranges of 65% ginger, 25% red pepper, 10% cloves, and 10% black pepper. The level of spices in the product is critical to consumer acceptance of the product (Anounye, 1997; Akoma, et al., 2002. This is because some of the added spices at high concentration make the product objectionable. Ihekuronye and Ngoddy (1985) and Adeyemi and Umar (1994) reported that clove contains large amount of volatile oils which could contributes to unacceptable strong flavor when added at high concentration. Spices added to *kununzaki* may also be a serious source of microbial contamination. These facts were substantiated by Akoma (1990), Onuarah, et al., (1987) as they reported the contamination of various spices with microorganisms.

2.2.0 Methods of *kununzaki* preparation

Akoma, et al., (2002) reported that at present two methods are used in the home preparation of *kununzaki*: traditional and improved traditional methods.

2.2.1 Traditional methods of *Kununzaki* fermentation

There are variations in the methods adoptable for the production of *kununzaki* from cereals, depending on the cultural background and people's food habit (Anounye, 1997). For instant among the Igala's of Kogi State, a brand called the Obilor is produced and consumed. It is a light brown gruel with sweet taste and low viscosity, prepared mainly from sorghum or millet or maize (Achi, 1990). Achi (1990) and Akoma et al., (2002) reported the production of this product as follows: a blend of sorghum and millet are soaked in water for about 17 to 24 hours. The grains were drained and re-soaked for another 15 minutes and after wrapped in fresh banana leaves. The grains were wet milled in to slurry in a 4:1 (g/g) ratio of sorghum and millet. Boiled water added to the slurry and the resulting mash cooled, filtered and residue discarded. The filtrate is then boiled again with continued mixing, and then filtered. The filtrate is further boiled again for 30 minutes, and then cooled rapidly. The problems associated with this method are the long time (several days) to produce and the final product, which is unacceptable to some ethnic groups. Also as cereals are the main ingredients of production, they

have low protein content and the product produced out of them are even lower due to the methods of processing (Goji, et al., 2000).

Akoma, et al., (2002) described two methods of *kununzaki* preparation. In one of the methods, the cereal grains are soaked in water for about 24 hour at room temperature, wet milled and sieved. The resulting slurry is divided in two unequal portions; one portion is cooked by the addition of hot water and then mixed with the uncooked portion (being the source of inoculums) and allowed to ferment for about 8 hours. In the second method, a portion of the cereal are malted, dried and ground before mixing with uncooked portion and stirred vigorously before allowing fermenting for 8 hours. The hydrolytic enzyme (amylase) in the malted grain aids in digesting the thick slurry thereby converting the complex carbohydrates molecules to simple sugars. The final product is usually sweet and therefore, may not require the addition of granulated sugar. Onuarah, et al., (1987) describe a method where the grains for the production of *kununzaki* were soaked in water for 1-2 days at ambient temperature and then wet milled into slurry. Adding cold water to a portion of the slurry made a paste of the slurry. Boiled water was added to the second portion of the slurry to gelatinize the starch and form a homogeneous mixture. The remaining portion of the paste was subsequently mixed with the homogeneous portion. The mixture was then cooled and sieved. A composite spice powder (ginger, black pepper, cloves and red pepper) mixed with water and passed through

a muslin cloth or metallic sieve was added to the homogeneous mixture. The proportion of the spices added were 65%, 25%, 10%, 10% for ginger, red pepper, cloves and black pepper respectively. Granulated sugar was added to the final product to taste. The methods reported by Efiuvweuwere and Akoma, (1995) involve '*kununzaki*' production by steeping 500g millet in 1liter water for 24hours at ambient temperature (27-34°C). The soaking water was decanted and the grains blended with 10g ground ginger in 0.2liter tap water, the slurry was then sieved through a mesh of approximately 350μm using enough tap water. Sedimentation was allowed for 3-5 hours at room temperature and the supernatant discarded. The remaining pasty and milky sediment was divided in to two portions. One portion was cooked at 45-50°C and then mixed in a 1:1 (v/v) ratio with uncooked portion. The mixture was diluted with tap water, allowing fermenting for 8hours and then sweetened with 2% (v/v) granulated sugar. Adeyemi and Umar (1994), describe a method where cleaned peal millet grains were steeped and wet milled in combination with spices. The resulting slurry was then sieved and allowed to settle after which the supernatant was decanted. The slurry was then boiled and then sieved with the uncooked portion before sieving. The beverage is sieved as *kununzaki*. Sopade and Kassum (1992) prepared *kununzaki* by steeping 250g of millet over night. The soaked millet was then washed and ground with 0.35liter of tap water in an attrition mill. About 0.75liter of water was later added and the slurry

sieved with muslin cloth. The filtrate was boiled for about 20 minutes with 1g of red pepper, 3.5g of ginger and 300g of sweet potato, which has been grounded in 0.3 liter of water prior to the addition of millet filtrate. The mixture was left standing to cool before sieving and addition of 200g granulated sugars.

Iyang and Dabot (1997) on the other hand, reported the use of guinea corn in *kununzaki* preparation. Some quantity (1kg) of red guinea corn (*Sorghum bicolor)* cleaned and malted by steeping in water for 24 hours at room temperature. The water was drained and the grains spread on a clean jute bag and allowed to germinate at room temperature. Germination was for 48 hours with intermittent sprinkling of water. The sprouted grains were sun dried or oven dried at 50°C to a constant weight. An un-malted portion (1.6kg) of sorghum grains was wet milled and made in to slurry. Adding boiling water continuously to the paste with constant stirring to form a viscous semi liquid product gelatinizes the slurry. This was allowed to cool to about 50-60°C after which slurry from the malted sorghum flour prepared by adding minimal amount of water is added, before mixing thoroughly and sieved. The filtrate was then allowed to ferment at room temperature for 12-18 hours before sieving.

2.2.2 Improved Method *Kununzaki* Preparation by the addition Soybeans

The usefulness of legumes in the developing high protein foods to meet the needs of the vulnerable group of the population is now well recognized, and several high protein foods have been developed industrially, in different parts of the developed World. Such foods are assumed nutritionally well balanced and have acceptable texture and flavor. Most of them are precooked, roller dried mixture based on blend of cereals-legumes or other protein rich seeds (Nuru, 1985; Nkama, 1993). Alternative to the industrial processing of the high protein foods that would meet the needs of all vulnerable groups in Africa seems to be the increased use of improved traditional food preparation technology (Nkama, 1993, CFP, 2005). The advantages in the improvement of the traditional food preparation technology are that since the process is already known, problems associated with its correct application will be minimal and easier to communicate to the end-users. Equipments for the process may be available locally, and this will eliminate problems of spending hard earn foreign exchange for equipment procurement (Safa-Dedeh, 1984). The final product also will be acceptable to the people since it is their food.

In order to improve the nutritive values of *kununzaki*, the traditional methods earlier outlined that involves the use of only cereal grain was modified with

addition of soybean. In this new technology, cleaned millet grains were steeped in water over night. Sorted soybeans were blanched for about 30minutes and steeped over night. The steeped cereal and legume were washed after standing overnight, spices- ginger, black or red pepper, cloves and cinnamon were added to a 4:1 (g/g) measure of millet and soybeans. The grains were wet milled and about 2-3 liters of water is added during the milling from paste to form slurry. About twice the volume of boiling water is added after dividing the slurry into 2:1 portion. The 2 portions were cooked by the addition of hot water to gelatinize the starch and turn the slurry in to mash. Raw slurry is then added after cooking, before allowing standing for about 2-3 hours to cool before sieving and sweetening with sugar to taste. The improve method has the advantages of having high protein in the final product, short production steps and fermentation period. This short fermentation time prevent deterioration of soybeans protein. The soy-*kununzaki* is usually sold in pet bottles or polythene bags, and mostly taken as appetizer or thirst quencher. It is a very important weaning food, and used extensively during the hot period in the north, and during the Muslim fasting period of Ramadan.

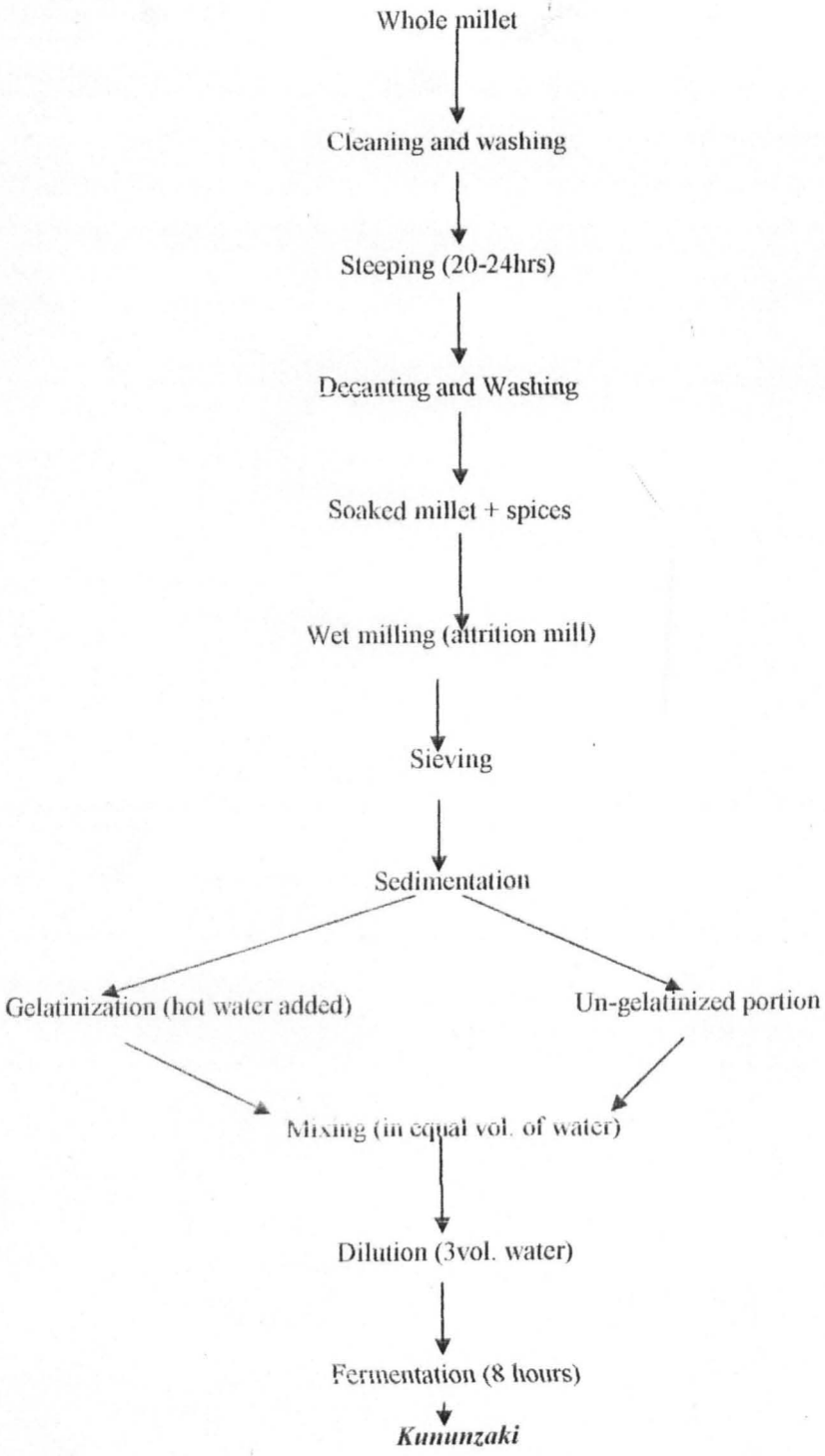

Figure 1: Traditional method of kununzaki processing (Efiuvweuwere and Akoma, 1995)

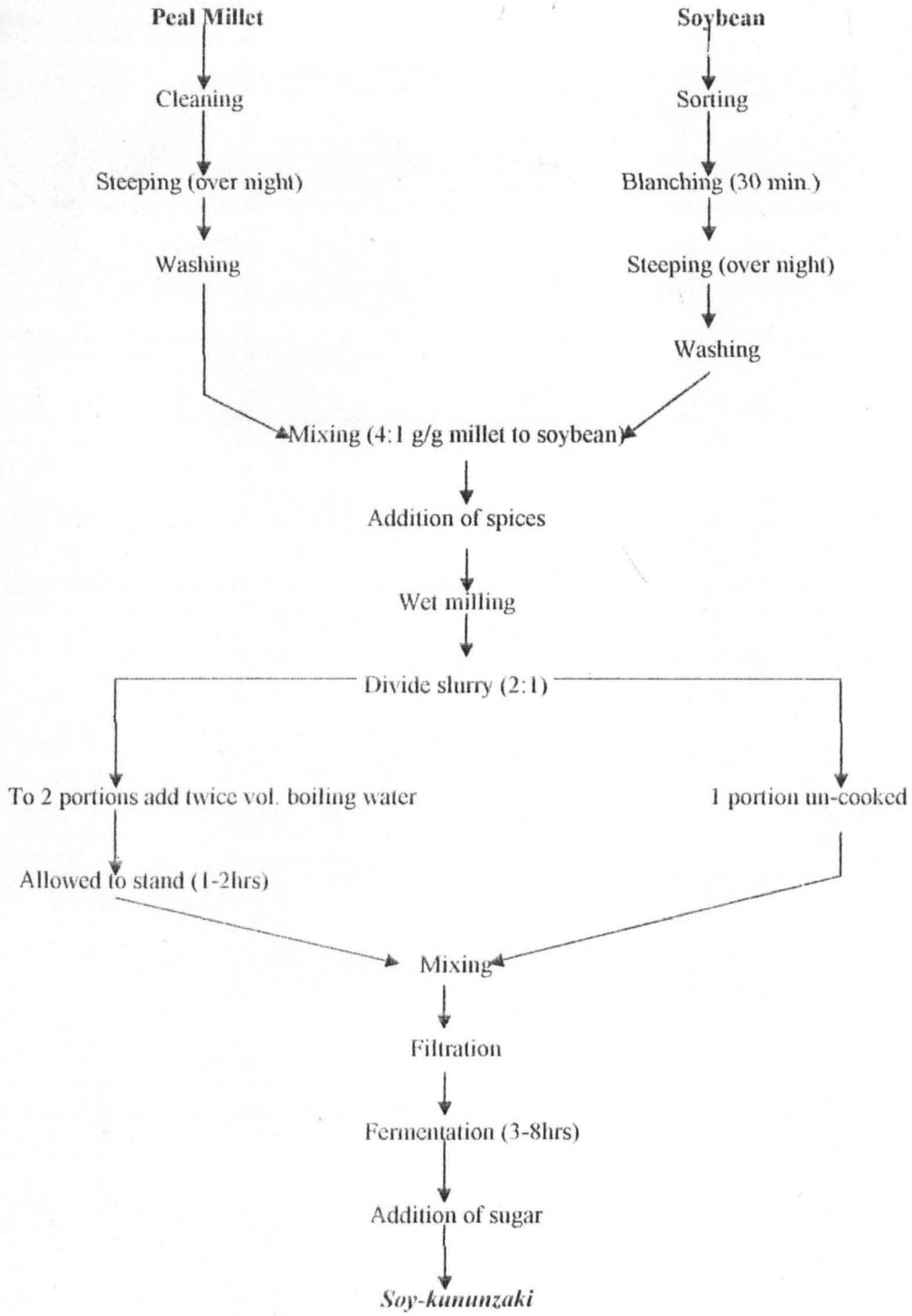

Figure 2: Improved method of *kununzaki* processing with addition of soybeans.

Sources: Anounye 1997; Bankole and Olatunji 2001

2.3.1 Microbiology of *Kununzaki* Production

The establishment of micro flora of a given raw material or food product its self is necessary because of the safety hazards related and attributed to food related illness (Jideani, et al., 2001). Bryan (1988) advocated an intensified research into the microbial quality and safety hazards associated with ethnic foods as people migrate from one part of a country to the other. While Jideani, et al., (2001) called for accelerated effort towards development of cheap, adoptable and sustainable quality control protocol for locally produced beverages and street vended foods. The diversity of microorganism and maximum counts obtained from street foods reflect the microbial quality of the ingredients used and the personnel in the production of such foods (Goji, et al., 2000).

Though information on the microorganisms involved in the carbonated beverages exist, those on *kununzaki* are scanty in literatures, Bryan, et al., (1992) reported that street vended foods have shown epidemiological links with food related illness and laboratory results have shown high microbial count and the presence of food borne pathogens on street vended foods. Ilori, et al., (1991) had reports indicating that fungi and molds cause the most serious problem in carbonated beverages, while in beer, bacteria and fungi predominates.

Akoma (1990) reported the isolation of *Bacillus macerans, Enterobacter cloacae, Enterobacter aerogenes, Lactobacillus famentum, Lactobacillus leichmania,*

Thanmidium spp, Fusarium sporotidoids and *Aspergillus flavus* from unfermented millet. He also reported the isolation of *Bacillus subtilis, Bacillus macerans, Enterobacter chacae, Aspergillus spp* and *Rhizobium nigricans* from dried ginger. Patel, et al., (1976), Mason (1979), Onuarah, et al., 1987) and Efiuvweuwere and Akoma (1995 and 1997) all reported that ginger, black pepper, cloves and red pepper are contaminated by *Staphylococcus spp* and *Corynebacterium.* Ilori, et al., (1991) and Efiuvweuwere and Akoma (1995) in a separate studies of the effect of malting and brewing on the microorganisms associated with sorghum grain and malts and the microbiology of the product during fermentation respectively reported the presence of *Escherichia coli, Enterobacter cloacae, Pseudomonas aerogenes, Sarcin, Bacillus cerus, Klebsiella aerogenes* and *Lactobacillus, A. flavus, Rhizopus stolonifer, Penicillum, Fusarium* and *Aspergillus niger.* Reports by the International Commission on Microbiological Specification of Foods (ICMSF, 1980) and Jay, (1980) revealed that ingredients such as ground cereal flour used in preparing cereal based meals contain high proportion of both spore formers especially *Bacillus, Lactobacillus planetarium, Streptococcus lactis* and yeast occur. Efiuveweuwere and Akoma (1995) also reported the isolation of *Lactobacillus, Sacharomyces cerevisiae* and some strains of pathogenic *Bacillus* and mycotoxic *Aspergillus* as associated with the production of *kununzaki.* Hesseltine, et al., (1966) were not able to isolate aflatoxin on soybeans inoculated

with *Aspergillus flavus*, although aflatoxins are not considered a problem in soybean, Farag, et al., (1986) detected aflatoxins in sterilized soybeans inoculated with *Aspergillus parasiticus*. Fungi known to damage soybeans includes *Diaporthe phaseolorum*, *Collectotrichum dematium* var. *truncate* and *Cercospora kikukii*, which found their way into products, produced from legume (McDonal, 1985).

2.3.2 Dynamics of microbial succession during *kununzaki* fermentation

The dynamics of microbial succession in any food matrix is complex. The microbial ecology of the matrix reflects the behavior and survival of some microorganism (Anon, 1980; Efiuvweuwere and Amadi 1992) and the death and disappearance of others. During the late stage of the fermentation, there was a correlation between titratable acidity and the lactic acid bacterial species (Efiuvweuwere and Akoma, 1995). Total viable count gradually increased during the production process up to the paste formation stage, and these fell significantly after the mixing stage and eventually become negligible. *Lactobacillus* gradually increased in number through out the production process. The microbial flora was generally dominated by *Lactobacillus fermentum*, *Lactobacillus leichmammi*, *Bacillus subtilis*, *Enterobacter aerogenes* and *Enterobacter eloacae*, but the later disappeared during the later stages of the production. Growth of *Sacharomyces cerevisiae* became apparent with increased acidity. Anon (1980) that *Aspergillus*

niger was the most consistently isolated mould but *Saccharomyces cerevisiae* was only isolated during the final stages of fermentation as reported these authors. Efiuvweuwere and Akoma (1995) reported that at the beginning of *'kununzaki'* production, microbial population is initially seen to be mediated by diverse microbial flora (with approximately three bacteria to every two fungal cell) which eventually become gram positive flora (a reflection of many African fermented foods) Odunfa, 1985). Although, the apparent dominance by the *Lactobacillus* and the concomitant marked increase in acidity may provide some assurance of microbiological safety of the product, this may be doubtful since very high total viable count populations exceeding maximum levels (log_{10} 4.3) usually recommended for pasteurized liquid foods (Banwart, 1979, Gilbert, et al., 2000, WHO, 2005).

2.4.0 Chemical changes during *kununzaki* fermentation

The PH of *kununzaki* remains relatively high during the production process until after the second hour of fermentation when significant decrease occurs. There was also concomitant increase in the titratable acidity in all the treatments, however, the change were more prominent in those substrates treated with extracts from ground malted cereal (Efiuveweuwere and Akoma, 1995; Akoma et al., 2002).

2.5.0 Principles and Application of Hazard Analysis Critical Control Point (HACCP) system in food preparation

HACCP is a systematic approach to identification, evaluation and control of food safety hazards. It has its roots in the U.S. aerospace industry, and was developed by the Pillsbury Company in 1959 to ensure the safety of food in the new U.S. space program. Because the lives of the astronauts who developed food poisoning in the space would be in serious danger, NASA requested the creation of a preventive process to guarantee the quality and purity of food. HACCP was the answer (Weeks and Bagain, 2000). HACCP was first described publicly in 1971 at the National Conference on Food Protection (Shanaghy et al., 1993). After a public out cry following botulism out break involving canned soups, the U.S. Food and Drug Administration (FDA) mandated the first use of the HACCP by regulation in 1973 for all low-acid foods (Bryan, 1992). Reviewing the successful implementation of this regulation, the National Research Council of the United State in 1985 reported in favor of the process. The new report broke new ground by recommending wide spread use of HACCP in all food groups, including meat and poultry, sea foods, diary products, fruits and vegetables, beverages and more. The council also calls for the extension of the process to the food-service industries. Growing public awareness of the threat of microbiological pathogens in food and deaths from out breaks of food-borne illness, such as one involving

hamburgers tainted with *E. coliO*157.H7 at restaurant in the pacific Northwest in late 1992, prompted additional U.S. federal action. The U.S. Department of Agriculture (USDA) embraced HACCP as a science-based alternative to final product sampling technique. FDA announced plans in 1995 for hazard analysis critical control point implementation for all fruits and vegetable beverages, and is now considering establishing HACCP as the food safety standard through out all segments of the food industry under its authority (McDonough, 2003). Since the adoption of HACCP in food safety management, it has produce measurable and positive results across a spectrum of differing work situations and environment. Both the United State Centers for Disease Control and Prevention (CDP) and the USDA have released data showing progress in reducing food-borne pathogens, which are estimated to cause 76 million illness and 5,000 deaths per year (USCDP, 2002). Their 2001 FoodNet data show that the estimated incidence of infections caused by four key pathogens (*Campylobacter, Escherichia coliO1157:H7, Listeria,* and *Salmonella)* was 21% lower than in 1996 (USDA, 2000). The USDA program, prior to 1996 HACCP implementation, established baseline prevalence levels for the presence of microbial organisms such as *Salmonella* in meat and poultry. USDA data released in 2000 and 2001 showed significant reductions in bacterial levels across a variety of food products following HACCP implementation (USDA, 2002). Numerous academic researchers have also found

vidence documenting the usefulness of HACCP in reducing the levels of food borne pathogens in food production and food services (USGAO, 2001). While HACCP shifts a greater inspectional burden to food processors, the FDA says this reinforces industry's responsibility to make safe foods. In a January 2001 report, the U.S. General Accounting Office (GAO) cited deficiencies in the FDA's sea food HACCP program, including lack of attention to violation and lack of quantifiable data to access program effectiveness (VANCPS, 2001) and made available at http://www.patientsafety.gov?HFMEA.html. Officials of FDA noted substantial challenges in regulating seafood versus canned foods and meat products due to the wider variety of species and greater diffusion of processing and distribution, and more numerous critical points. In spite of setbacks, HACCP is here to stay, and is becoming an international requirement for safe food preparation and elimination of food safety hazards. Food safety hazards are physical, biological and chemical agents that are reasonably likely to cause illness or injury in the absence of their control. A HACCP system is a preventive system of hazard control rather than a reactive one. HACCP system is designed to prevent the occurrence of potential food safety problems caused by pathogenic microbes (Bryan, 1990a and 1990b; Byerklie, 1992). This is achieved by the assessment of the inherent hazards attributed to the product or a process, determining the necessary steps that will control the identified hazards, and implementation of

active managerial control practices to ensure that the hazards are eliminated or minimized (Bryan, 1988). Essentially, HACCP is a system that identifies and monitors specific food borne hazards that can adversely affect the safety of food product. This hazard analysis serves as the basis for establishing critical control points (CCPs). CCPs identify those points in the process that can be controlled to ensure the safety of the foods (Bryan, 1988; Bryan, 1990a; and FDA, 2004).

After 30 years since its conception, the HACCP has grown to become the universally recognized and accepted method of safety assurance (FAO, 1998). The International Standard Organization (ISO 9000; quality assurance) provides an excellent framework for the inclusion of HACCP principles and an ongoing basis for continual improvement (FAO, 2004). The recent and growing concern about food safety from government agencies, food industries and consumers worldwide has been the major impetus for the application of HACCP system. This concern has been substantiated by a significant increase in the incident of food borne disease in many countries of recent (WER, 2004). World Health Organization has recognized the importance of the HACCP system for prevention of food borne diseases for over 20 years and has played an important role in its development and promotion (WHO, 2005). The implementation of HACCP continues to evolve as hazards and their control measures are clearly defined. To meet the challenges presented by advances in food research all over the World, product development, and their impact on

ally produced ethnic foods, regulatory agency and the food experts must keep emselves informed. Food protection publications issued by the food industries, ofessionals' organizations, and other groups, continuing education programs can e particularly helpful in providing and understanding of food operations and how he application of HACCP can bring a focus to food safety that traditionally roduced foods lacked (FDA, 2004). The United State Federal Department of Agriculture has issued guideline to industries voluntarily interested in application of HACCP principles in food establishments. The document entitled 'managing food safety: A HACCP principles guide for operators of food services, retail food stores, and other food establishment at the retail level' is discussed at the web site *http://vm.cfsan.fda.gov/-dms/hret-toc.html*. The guide set up a framework for the retail food industries to develop and implement a sound HACCP food safety management system. The application of hazard analysis critical control point to food production was first pioneered by the Pillsbury Company with the cooperation and participation of the National Aeronautic and Space Administration (NASA), Natick Laboratory of the U.S Army, and the U.S Air Force Laboratory Project Group. Application of the techniques in the early 1960s created food for the United State space program that approach 100% assurance against contamination by bacterial and viral pathogens, toxins, and chemical or physical hazards that could cause illness or injury to astronauts. HACCP replace end-product test to prevent

od safety hazards and provide a preventive system for producing safe food that ad universal application (FDA, 2001).

2.5.1 Advantages of Hazard Analysis Critical Control Point in food Quality Control

The FDA, WHO, ISO, Codex alimentarius, and other regulatory agencies recommend the implementation of HACCP in food establishment, because it is a system of preventive approach to hazards control that is the most effective and efficient way to ensure food produced are safe for human consumption. HACCP system will emphasize the industry's role in continuous problem solving and prevention rather than relying solely on periodic facility inspections by regulatory agencies (FDA, 2004).

HACCP offers two additional benefits over the conventional inspection techniques. First, it clearly identifies the food establishment as the final party responsible for ensuring the safety of the food it produces. HACCP requires the food establishment to analyze its preparation method in a rational and scientific manner in order to identify critical control points and to establish critical limits and monitoring procedure (Bryan, et al., 1992). Secondly, an HACCP system allows the regulatory agencies to comprehensively determine an established level of compliance. Using a conventional inspection that does not provide a 'snap short' of the conditions at the

oint of data collection. However, by adopting an HACCP approach, both current nd pass conditions can be determined (Kemp, 1991). Traditional inspection is elatively resource-intensive and inefficient and is reactive rather than preventive compared to the HACCP approach for ensuring food safety. Regulatory agencies are challenged to find new approaches to food safety that enables them become more focused and efficient and to minimize costs when ever possible. Thus, advantages of HACCP-based inspection are becoming increasingly acknowledged by the regulatory agencies (ICMSF, 1986, 1989). In a research project conducted and coordinated by the United State Federal Department of Agriculture (FDA) and a number of State and local jurisdictions in the U.S.A., and collaboration with two volunteer food stores and restaurants. It was demonstrated that HACCP is a viable and practical option to improve food safety. FDA believes that HACCP concepts have matured to the point at which they can be formally implemented for all food products in an industry-wide basis (Stevenson, 1990).

2.5.2 Development of hazard analysis critical control point principles

In November 1992, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) of the U.S Department of Agriculture defined seven (7) wide acceptable HACCP principles that were to be considered when developing a

ACCP plan. In 1997, the NACMCF reconvened the HACCP group to review the mmittee's November 1992 HACCP document and to compare it to current ACCP guidelines prepared by the CODEX committee on food hygiene. From ese review, HACCP was defined as the systematic approach to the identification, valuation and control of food safety hazards based on the following seven rinciples (FDA, 2001).

Principle 1: Conduct a hazard analysis

Principle 2: Determine the critical control point (CCPs)

Principle 3: Establish critical limits

Principle 4: Establish monitoring of procedure

Principle 5: Establish corrective action

Principle 6: Establish verification procedure

Principle 7: Establish record keeping and documentation procedures

Principle 1: Hazard Analysis

Purposes

The hazard analysis process accomplishes three purposes: hazards of significance are identified, provide a risk basis for selecting likely hazards, and identified hazards can be used to develop preventive measures for a process or product to ensure or improve food safety. The first step in the development of a HACCP plans for a food operation is identification of hazards associated with the product and its raw materials. A hazard may be chemical, physical, or biological property that can cause food to be unsafe. The analysis of hazards requires the assessment of two factors with respect to any identified hazards, i.e. the likelihood that the hazard will occur and the severity if it does occur. It also involves establishment of preventive measures for control. Hazards that involve low risk and that are likely to occur need to be considered for the purpose of HACCP. To be effectively addressed, hazards must be such that their prevention, elimination or reduction to acceptable level is attained (Foster, 1989; Buchaman, 1990; Corlett, 1991 and Sanni, 1997). Numerous issues have to be considered during hazards analysis. These relate to factors such as ingredients, processing procedure, distribution and the intended use of the product (Sanni, 1997). These issues include whether a food contains sensitive ingredients that may create microbiological, chemical or physical

hazards, whether sanitation practices that are used can introduce these hazards to the food that is being prepared or processed (Buchaman, 1990). An example is whether the consumer before consumption will heat the finished food product, if it is consumed off the premises. Even factors beyond the immediate control of the food producer, such as how the food will be treated after taken out by the consumer and how it will be consumed, must be considered because these factors could influence how food should be prepared or processed in the establishment (Foster, 1989; Corlett, 1991 and Sanni, 1997).

Flow diagram

Consequently, a flow diagram that delineates the steps in the process from receipt of raw materials forms the foundation for the application of the seven principles. The significant hazards associated with each step in the flow diagram should be listed along with preventive measure proposed to control them. This tabulation will be used under principle 2 to determine CCPs (Foster, 1989). The flow diagram should be constructed by team that has the knowledge and expertise on the product, process and the likely hazards (Corlett, 1991; FDA, 2005).

Microbiological Hazards

Food borne biological hazards includes bacteria, virus, and parasitic organisms. These organisms are commonly associated with raw materials entering the food

production chain (ICMSF, 1986; FDA, 2005; Sanni, 1997). Many of these pathogens occur naturally in the environment where foods are grown. Most are killed or inactivated by adequate cooking and numbers are kept to a minimum by adequate cooling during distribution and storage. Bacterial pathogens comprise the majority of reported food borne disease out breaks and cases. A certain level of pathogens may be expected with some raw materials. Temperature abuse such as hot or low holding temperature can significantly magnify this number. Cooked food, which has been subjected to cross contamination with pathogens often, provides a fertile medium for their rapid and progressive growth (Rhodes, 1991; Gilbert, et al., 2000). Enteric viruses can be food borne, water borne, or transmitted from person or from animals. Unlike bacteria, a virus cannot multiply out side of a living cell. Hepatitis A and Norwalk viruses are examples of viral hazards associated with ready-to-eat foods (Gilbert, et al., 2000). Parasites are most often animal or plant specific and can invade humans in their life cycles. Parasitic infections are commonly associated with under cooked meat products or cross contamination of ready-to-eat foods. Food borne parasites in foods that is intended to be eaten raw, marinated or partially cooked can be killed by effective freezing techniques (Gilbert, 2000; FDA, 2001). The following organisms and factors are considered in the assessment of severity of biological hazards, which may be associated with foods being prepared, served or sold as street vended foods

(ICMSF, 1986). Severe hazardous microorganisms and parasites are: *Clostridium botulinum* types A, B, E and F, *Shegilla dysenteria, Salmonella typhi, S.paratyphi A* and *B, Hepatitis A* and *E, Brucella abortus, B. suis, Vibrio cholerae, V.vulnificus, Taenia solium* and *Trichinella spiralis.* Moderately hazardous organisms are: *Listeria monocytogenes, Salmonella, Shigella, enterovirulent Escherichia coli, Streptococcus pyogenes, Rotavirus, Norwalk virus* group and other parasites, *Entamoeba histolytica, Diphyllobothrium latum, Ascaris lumbricoides, and Cryptosporidium parvum.* Although classified as moderately hazardous, complications may be severe in certain susceptible population. Moderately hazardous group that are classified as of limited spread are: *Bacillus cereus, Campilobacter jejuni, Clostridium perfiringens, Staphylococcus aureus, Vibrio parahaemolyticus, Vibrio cholerae, Yersinia enterocolitica, Giardia lamblia* and *Taenia sagnata*

Chemical hazards

Chemical hazards in foods should be considered during a hazards analysis. Chemical contaminants may be natural occurring or may be added during food processing and storage. Storage chemicals at certain levels have been associated with acute cases of food borne illness and can be responsible for chronic illness at lower levels (FDA, 2001). The following are examples of chemical hazards found

within the naturally occurring category, Mycotoxins (eg aflatoxin) from moulds, Scombrotoxin (histamine) from protein decomposition, Ciguatoxin (from marine dinoflagellate), Toxic mushroom species, Shellfish poisoning, Diarrhetic shell fish poisoning, plant toxins etc. And added chemicals are: pesticides, fungicides, fertilizers, insecticides, antibiotics, and growth hormones. Others are polychlorinated biphenyls, industrial chemicals, prohibited substances, toxic elements and compounds (Lead, Zinc, Arsenic, Mercury and Cyanides (Pierson and Corlett, 1992).

Food allergens

Each year the Food and Drug Administration of the United State Department of Agriculture (recently the National Agency for Food and Drug Administration and Control (NAFDAC) in Nigeria) receives reports from consumers who experienced adverse reactions following exposure to allergenic substances in foods. Food allergies are abnormal responses of the immune system, especially involving the production of allergen-specific immunoglobulin (I_gE) antibodies, to naturally occurring proteins in certain foods that most individuals can eat safely. Frequently, such reactions occur because of the presence of the allergic substances in the foods that are not declared on the food label (FDA, 2001). To combat this problem, the Food and Drug Administration issued a letter titled 'Notice to Manufacturers',

dated June 10, 1996, which addressed labeling issues and Good Manufacturing Practices (GMPs). This letter is available on FDA's website, *www.cfsa.fda.gov-lrd/allerg7.html*. The agency believes that there is scientific consensus that the following foods can cause serious allergic reaction in some individuals and account for more than 90% of all food allergies (Sampson, 1997). Soybeans, Peanut, Milk, Eggs, Fish, Crustacean, Tree nuts, and Wheat are most reported food allergies (Sampson, 1999; FDA, 2001).

Physical hazards

Illness and injury can result from hard foreign objects in foods. These physical hazards can result from contamination and/or poor procedures at many points in the food chain from harvest to consumption including those within food establishment (Pierson and Corlett, 1992; Sanni, 1997).

Hazard analysis process

This point in hazard analysis consists of asking a series of question, which are appropriate to each step in the flow diagram. The hazards analysis should question the effect of a variety of factors upon the safety of the food.

Ingredients: Does the food contain any sensitive ingredients that are likely to present microbiological hazards, (*Salmonella Typhi, Staphylococcus aureus)*,

chemical hazards aflatoxins, antibiotics or pesticide residues) or physical hazards (stones, glass, bone, metals etc)

Procedure used for preparation/processing: Does the preparation procedure or process include a controlled step that destroys pathogens or their toxin? Is the product subject to recontamination between the preparation steps (for instant, cooking) and packaging? (FDA, 2001).

Microbial contents of the food: is the food commercially sterile (low acid foods)? Is it likely that the food will contain viable spore forming or non forming pathogens? Does the microbial population change during and after processing? Does this change microbial population alter the safety of the food? (Stevenson, 1990).

Principle No 2: Identification of Critical Control Points (CCPs).

A CCP is a point, step or procedure at which control can be applied and a food safety hazards can be prevented, eliminated, or reduced to acceptable levels. For examples cooking or refrigeration. Cooking that must occur at specific temperature and for a specified time in order to destroy a microbiological hazard is a CCP. Likewise, refrigeration or the adjustment of a food pH to a level required preventing hazardous microorganisms from multiplying or toxins from forming are

also CCP (Stevenson, 1990; Summer, et al., 1992). Many points in food preparation may be considered as CCP, but very few are actually CCPs. Concerns that do not impact safety may be addressed as CCPs, however, since these control points do not relate to food safety, they are not included in the HACCP plans (FDA, 2001). CCPs must be carefully developed and documented. In addition, they must be used only for the purposes of product safety (Weeks and Bagian, 2000).

Principle No 3: Establishment of Critical limits

This step involves the establishment of a criterion that must be met for each preventive measure associated with a CCP. Critical limit could be thought of as boundaries of safety for each CCP and may be seen as preventive measures such as temperature, time, physical dimension, water activity, pH, and available chlorine. Critical limits are usually derived from sources such as Regulatory Standard Guideline, scientific literatures, experimental studies, and consultation with experts (USGAO, 2001; USDA, 2002). Other criteria frequently used are titratable acidity, humidity, salt concentration, and viscosity (FDA, 2001).

Principle No 4: Establishment of procedure to monitor CCPs

Monitoring is a planned sequence of observation or measurement to assess whether a CCP is under control and to produce an accurate record for uses in future verification procedures. There are three main purposes for monitoring:

1. It tracks the system's operation so that a trend toward a loss of control can be recognized and corrective action can be taken to bring the process back to control before a deviation occur (USGAO, 2001).

2. It indicates when loss of control and a deviation have actually occurred, and corrective action must be taken; and

3. It provides written document for use in verification of the HACCP plan (FDA, 2001, USGAO, 2001; USDA, 2002).

An unsafe food may result if the process is not properly controlled leading to a deviation. The result of these potentially serious consequences of a critical defect monitoring procedures there must be an effective monitoring system (USDA, 2002). Some foods containing microbiologically sensitive ingredients may not be an alternative to microbiological testing. For this reason, microbiological testing has limit in a HACCP system, but is valuable as a means of establishing and verifying the effectiveness of control at CCPs (FDA, 2001).

Principle No 5: establishment of corrective action to be taken when monitoring shows that a critical limit had been exceeded

Although the HACCP system is intended to prevent deviation from occurring, perfection is rare, if ever, achievable. Thus, there must be a corrective action plan in place to:

1. Determine the disposition of any food that was produced when a deviation was occurring;
2. Correct the cause of deviation to ensure that the critical control point is under control and;
3. Maintain records of corrective actions (Rhodes, 1991).

Because of the variations in CCPs for different food operations and the diversity of possible deviation, specific correction action plans must demonstrate that CCP has been brought under control. Individuals who have a thorough understanding of the operation, products, and HACCP plan must be assigned responsibility for taking corrective action. Corrective action procedures must be documented in the HACCP plan (Weeks and Bagain, 2000).

Principle No 6: Establishment of procedure to verify that the HACCP system is working

The first phase of the process is the scientific or technical verification that the critical limits at CCP are satisfactory. This can be complex and may require intensive involvement of highly skilled professionals from a variety of disciplines capable of doing focused studies and analysis. A review of the critical limit is necessary to verify that the limits are adequate to control the hazards that are likely to occur (FDA, 2001). The second phase of the verification ensures that the facility's HACCP plan is functioning effectively (Gilbert, et al., 2000). Functional HACCP systems require little end product sampling; since appropriate safeguards are build in, early in the preparation. Therefore, rather than relying on end product sampling, food establishment must rely on frequent review of CCP records, and determine that appropriate risk management decision and product disposition are made when preparation deviation occur (Gilbert, et al., 2000; FDA, 2001; ISGA, 2002).

Principle No 7: Establishment of effective record keeping system that documents the HACCP system

The last principle requires the preparation and maintenance of a written HACCP plan by the food establishment. The plan must detail the hazards of each individual

or categorical product covered in the plan. It must clearly identify the CCP. CCP monitoring and record keeping procedures must be shown in the establishment's HACCP plan (Stevenson, 1990; FDA, 2001; ISGA, 2002). The principle requires the maintenance of records generated during the operation of plan. The record keeping associated with HACCP procedures ultimately makes the system work. One conclusion of the study of HACCP performed by the U.S Department of Commerce is that correcting problems without record keeping almost guarantees that the problem will recur. The requirement to record events at CCPs on a regular basis ensures that preventive monitoring is occurring in a systematic way. Usually, occurrences that are discovered as CCPs are monitored or that otherwise come to light must be corrected and recorded immediately with notation of the corrective action taken (ISGA, 2002). Hazard analysis critical control point (HACCP) check list plan as reported by International Sprout Growers Association (ISGA, 2002) and made available at the website http://www.isga-sprout.org.

Table 1: Hazard Analysis Critical Control Point (HACCP) checklist plan

Control point	Hazards	Control measures
Raw material: dried beans	Mold or bacterial growth due to damp storage conditions	Dry storage, humidity control and moisture control in beans
Dried beans	Contamination by bird, rodents or insects	Birds, rodent and insect control program
Dried beans	High microbial loading	Selection of supplies of good quality raw materials
Dried beans	Presence of pathogens on seed	Microbial test on dried beans
Dried beans	Contamination by foreign matter	Inspection, sieving, washing
Dried beans	Contamination of pesticides	Discard seed
Raw materials: packaging material	Contamination by birds, rodent or insects	Birds, rodents, and insects control program
Packaging material	Contamination by dirt	Clean storage environment
Soaking and germination of seed	Growth of surface microbial contamination	Surface decontamination of seed
Soaking and germination of seed	Contamination of soaking container	Cleaning and disinfecting of recycled soaking or germination container
Soaking and germination of seed	Contamination from water sources	Disinfections of water supply
Growth of seed sprout	Excessive microbial proliferation	Use of disinfected soaking water
Packaging	Contamination due to unsanitary handling practice	Personnel hygiene control, and sanitation of equipment
Storage	Microbial growth	Storage under chilled condition
Distribution	Microbial growth	Chill distribution chain
Consumer	Storage abuse of product leading to microbial growth	

ISGA, 2002, Available at http://www.isga-sprout.org

2.6.0 Evaluation of process capability of HACCP to meet the microbiological limit for food quality

Hazard analysis critical control point identifies specification hazards and preventive measures from their control. Its effect is to focus control at the CCPs, and so provide a basis on which the performance of a food production system and the expected safety of the food produced by it can be assessed immediately (CFP, 2004). Microbiological criteria for foods are limiting values (upper and lower limit) concerning the presence or number of specific microorganism, or quantity of their toxins, per unit mass, volume or area of specified food. They are particularly relevant for the verification of HACCP system (McDonough, 2002). Therefore to compare the out put of a process with the recommended specification limits and make a statement about how well the process can control or met the specification, we are often required to look at the process capability (C_p) (Anonymous, 2005). A capable process is one where almost all the measurements falls in side the specification limits (Sanni, 1997; Anonymous, 2005). This can be represented by the diagram below:

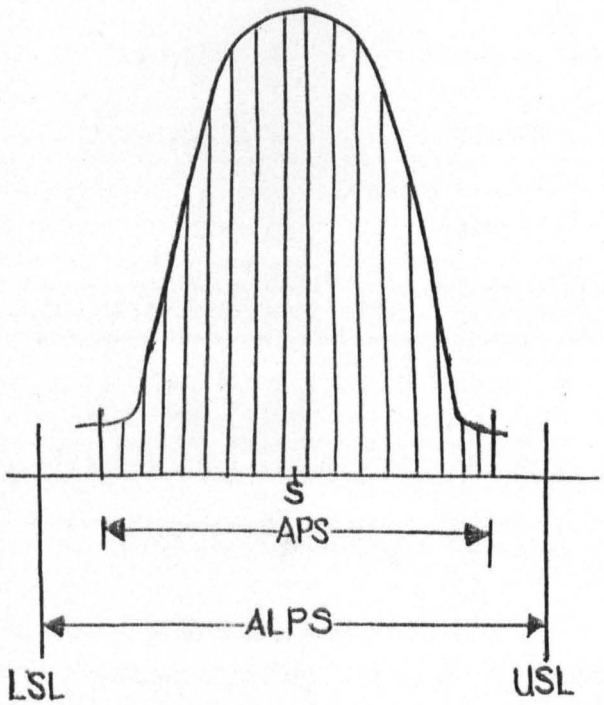

Figure 3: Process Capability (C_p) graph.

LSL = lower specification limit, **ALPS** = allowable process spread, **USL** = Upper specification limit **μ** = Population mean, **APS** = actual process spread, **X** = sample mean

There are several statistics that can be used to measure the process capability: C_p, C_{pk} and C_{pm}. The C_p, C_{pk} and C_{pm} statistics assumed that the population of the data value is normally distributed.

Case 1: Two-sided specifications

Assuming a two-sided specification (where there is upper and lower specification), if μ and ð are the mean and standard deviation respectively of the normal data and the USL, LSL and T are the upper, lower and target specification limits respectively, then the process capability indices are as follows:

$$C_p = USL - LSL / 6ð \quad \text{----------(1)}$$

$$C_{pk} = \min [USL - \mu / 3ð, \mu - LSL / 3ð] \quad \text{----------(2)}$$

$$C_{pm} = USL - LSL / 6\sqrt{ð2 + (\mu - T)\,2} \quad \text{----------(3)}$$

For the estimation of process capability using a sample data, estimators are usually indicated with 'hat' over them.

$$\hat{C}_p = USL - LSL/ 6ð \quad \text{----------(4)}$$

$$\hat{C}_{pk} = mim [USL - X / 3S, X - LSL / 3S] \quad \text{----------(5)}$$

$$\hat{C}_{pm} = USL - LSL / 6\sqrt{S^2 + (X - T)^2} \quad \text{----------(6)}$$

Where C_p, C_{pm}, C_{pk}, is process capability estimators and S, X is sample standard deviation and mean respectively. The estimator for C_{pk} can also expressed as:

$$C_{pk} = C_p (1 - k)^2 \qquad \text{----------(7)}$$

Where k is a scaled distance between the midpoint of the specification range, m, and the process mean μ denote the midpoint of specification range by

$$m = (USL + LSL) / 2 \qquad \text{----------(8)}$$

the distance between the process mean, μ, and the optimum, which is the m, is $\mu - m$, where $m \leq \mu \leq LSL$.

Case two: One-sided (unilateral) limit

There are many cases where only the lower or upper specifications are used. Using one specification limit is called unilateral or one-sided case. The corresponding capability indices are:

C_{pu} = allowable upper limit / actual upper limit spread

$$= USL - \mu / 3\delta \qquad \text{----------(9)}$$

And, C_{pl} = allowable lower limit / actual lower limit spread

$$= \mu - LSL / 3\delta \qquad \text{---------- (10)}$$

Where μ and ð are the process mean and standard deviation respectively. Estimators of $\mathbf{C}_{pu}$ and $\mathbf{C}_{pl}$ are obtained by replacing μ and ð by X and S, respectively. The following relationship holds:

$$\mathbf{C}_p = \{C_{pu} + C_pl\} / 2 \qquad \text{----------(11)}$$

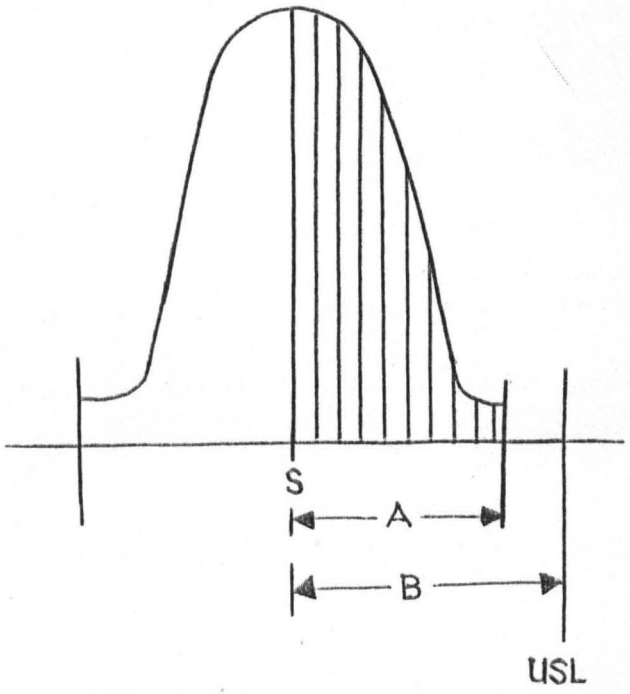

Figure 4: Normal distribution curves for unilateral quality process capability test

A = Actual process spread B = Allowable upper spread

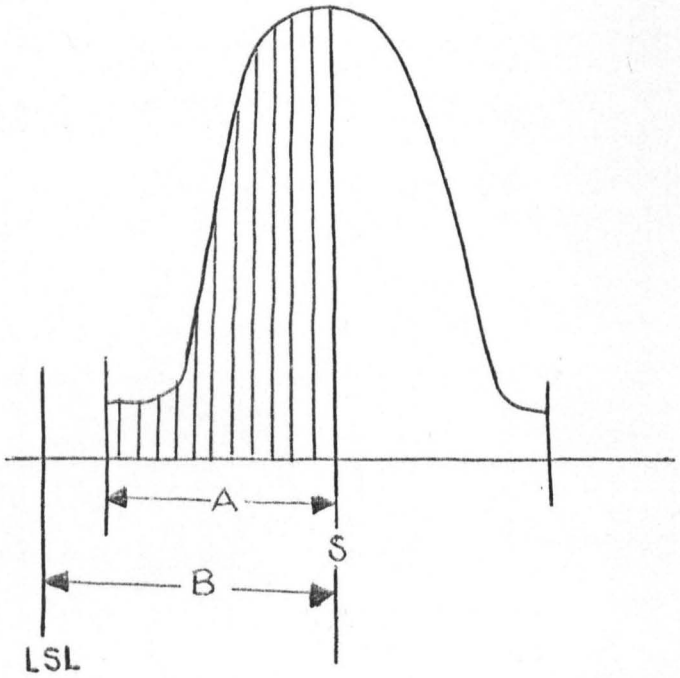

Figure 5: Normal distribution curve for unilateral (LSL) process capability test

A = Actual lower limit, B = allowable lower limit

The process by which we achieve safe food preparation is called quality control, so quality is the application of statistical principles and techniques in all stages of design, production, maintenance and services, directed towards satisfaction of demand (Solomon, 2001). A useful view from this is that, quality is introduced when a product is designed, created during its processing and verified by testing and control operations and it's apparent during its use.

2.7.0 Control of microorganisms using electrolyzed water (EW)

Continual technological break through in the field of electrolysis hold the promise to make electrolyzed water obtained from electrolysis of salt solution both a practical and economical process for a wide range of commercial, industrial, medical, dental and possibly drinking applications (Hung, 2004). Mahmond, et al., (2004) has reported that electrolyzed water produces the following effects:

1. Produces hyper oxygenated water, which kills bacteria, viruses and other biological contaminants in various medical and dental applications and provides significant and high beneficial effect to plants, animals and humans.

2. Cause organic and inorganic water borne contaminants to coagulate and precipitate out of solution, rendering them easy to remove with simple filtration; and

3. Breaks down harmful, toxic substances into non-harmful components.

A system of producing electrolyzed water for killing microorganisms while washing and U.S. fast food chains are trying soaking raw materials. The treated water has been shown to be effective in killing a range of poisoning bacteria including E. coli, *Salmonella* and *Listeria,* but does not affect the taste and appearance of the food (HSNI, 2000). In a study conducted by Park, et al., (2002) on the effectiveness of electrolyzed water as a sanitizer for treating different surfaces shows its effectiveness in killing *Entertobacter aerogenes* and *Staphylococcus aureus* in pure cultures. One milliliter of each bacterium was subjected to 9ml of the structured water and control water for 30 seconds. Inactivation (reduction of >9 log 10 CFU / ml) of bacterial pathogens occurred within 30 seconds after exposure to electrolyzed water containing approximately 25 or 50mg of residual chlorine per liter. The effectiveness of electrolyzed water in reducing *E. aerogenes* and the same worker also tested *S. aureus* on different surfaces. After immersion of the tested surfaces in the EW for 5 minutes with agitation, population of the organisms were reduced by $2\log_{10}$ CFU/CM2 and by 1.7 to 1.9 log 10 CFU/ cm2 for *E. aerogenes* and *S. aureus* respectively.

Tatsumi (2002) experiemented the control of microbial contamination by electrolyzed water in Tofu (a soybean product) manufacturing. In his work,

soybeans were soaked in three types of EW: acidic EW, at pH 2.1, 1185 mV oxidation-reduction potential (ORP) and 100ppm chlorine; alkaline EW at pH 11.7, -120mv (ORP); and a mixture (weak acidic electrolyzed water) of water at pH 6.5, 891mv ORP, and 50 ppm chloride. Microbial population of the water becomes negligible after only 30 minutes of treatment in acidic water and 1hour in the mixture, while physiochemical characteristics of the product (Tofu and milk made from the soybeans) thus treated with the water were not changed at all. But this author was careful in making this conclusion 'EW is, however, very unstable and should be prepared only at the time of use. More over, acidic and weak acidic EW are easily inactivated when polluted by organic matter; therefore, it is necessary to always protect them from pollution and to first wash the manufacturing lines or material with EW before use'. Zhaohui and et al (2002) conducted a similar research, and in a published work titled 'microorganism control in packaged Tofu manufacture with electrolyzed water'. EW was applied to control microorganisms during the soybean soaking process of packed Tofu processing. Acidic electrolyzed water (AEW) and alkaline electrolyzed water (KEW) were prepared by electrolysis of 0.075% sodium chloride solution. Mixed electrolyzed water (AEW and KEW) was also prepared by adjusting pH to 6.5. Sterilization effects during soybean soaking and quality of soybeans produced from soybeans soaked in the three type of electrolyzed water are analyzed. Results

indicates that microorganisms in soy beans can be effectively be sterilized by using AEW or mixed EW as soybeans soaking water, although the available chlorine concentration, which can be considered to reflect the electrolyzed water condition, decreased time dependent. About 300 CFU / g of microorganism were detected in the original soybeans. And no microorganisms were found in the water before soaking. The sterilizing effects of electrolyzed water under different soaking times were evaluated by microorganisms' analyses of soaked soybeans and wastewater after soybean soaking. AEW act as strong bactericide and decrease bacteria count by about $2\log_{10}$ CFU/g within 6minutes of soaking. Similar antimicrobial activity was observed in mixed EW. No detectable bacteria was found in the wastewater from AEW or mixed EW after soybean soaking. The number of bacteria in soybean soaked in sterilized water decrease initially but then increased after 1hour soaking. KEW soaking showed result intermediate between acidic, mixed EW and sterilized water. The reason for the initial decrease in number of microorganisms in soybean after soaking according to Venkitanarayanan, (1999) in alkaline EW and sterilized water can be considered to be a simple elution effect from the original soybeans. However, the decrease from KEW was higher than from sterilized water. KEW, which is considered to be an aqueous NaOH solution, act as sanitizer and reduces the attachment of microorganisms on soybeans, surfaces (Koseki and Itoh, 2000a). Basic pH and negative ORP decrease the number of aerobic bacteria

(Vankitanarayana, 1999). The sanitation effect, a basic pH value, and negative ORP result in alkaline EW having an antimicrobial activity (Zhao, 2002). However, the same author commented that the negative ORP in alkaline EW is unstable and gradually changes to a positive value. Alkalinity in EW is also transient; this may be as a result of reaction of NaOH with carbon dioxide (Koseki and Itoh, 2000b) from the atmosphere and/ or from soybeans dissolved out into the soaking water. Some of these materials might have brought the pH to weakly acidic values not only in KEW soaking but also in other tested sclutions (Kijimal, et al., 1997). AEW has high ORP and available chlorine (Nakagarawa, 1998; Len, 2000). The anti microbial effects of EW have been attributed to relationship among pH, ORP and / or available chlorine concentration (Kijimal, et al., 1997; Koseki and Itoh, 2000c). Several studies has indicated that the main factor in the elimination of microorganism is the available chlorine and that hydroxyl free radical produced from hypochlorous acid in available chlorine acts on microorganisms (Koseki and Itoh, 2000c; Koseki, 2001; Hung, 2004). High ORP (+1000mv to +1200mv) prevent microbes from growing (KOREN, 2005), while appropriate dissolved chlorine (-10 to -60ppm), work to effectively give microbes a fatal blow. The antibacterial spectrum is wide and the water is effective against various bacteria, including spores, fungi, acid-fast bacteria and viruses, making it an ideal disinfectant for various applications (KOREN, 2005).

2.7.1 Antibacterial spectrum of electrolyzed water

EW has been found to be effective in the control of the following microorganisms as reported by KOREN (2005) in introducing a product on hygiene and safety and made available at the KOREN web site http://www.korenDekaisu/electrolyzedwater.org/html. *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginusa, E. coli, Salmonella spp,* others are *Trophozoite microbe, Bacillus cereus, Mycobacterium tuberculosis,* other acid fast bacterium, *Candida albicans, Trichophyton rubrum,* other fungi, *Enterovirus, Herpesvirus and Fluvirus.*

2.7.2 Advantages of the use of electrolyzed water in microbial control during food preparation

Several advantages have been reported by various authors that are involved in the use of electrolysis in structuring of water for microbial control. Among this advantages are:

1. Wide antimicrobial spectrum: the anti microbial spectrum is wide, and the water is effective against various bacteria, spores, fungi, acid fast bacteria and viruses, making it an ideal disinfectant for various applications (Stan and Daeschel, 2002).

2. High safety: unlike other chemical disinfectants, this water does not create insensible bacillus, the electrodes do not generate ozone dependant emissions. No chlorine remains in the water after chemical reaction with organic compounds (Stan and Daeschel, 2002; KOREN, 2005).

3. Friendliness to human body: although, EW is strongly acidic, or basic it does not cause chapped hands, so, it can be safely used for disinfecting human hands and fingers (Hung, 2004).

4. Adaptability and cost: the use of the technology is easy and its adoption will be easy too. The cost of production is low since only small amount of salt (just a few grams) if require and low voltage battery.

Electrolyzed water produced by applying an electric current to a very dilute salt solution, kills bacteria on fresh produce more effectively in some cases than heat or water containing chlorine, according to a research conducted by Yen-con Hung and presented as a report during the 220th national meeting of the American Chemical Society held on the 28th August, 2000 (Hung, 2000). The electrolytic process produces very acidic water. Hung believes the water's low pH (acidity) and potential for oxidation-reduction contributes to its effectiveness. Essentially, oxidation-reduction involves the exchange of electrons. In the case of bacteria like *E. coli*, *Salmonella* and *Listeria*, these exchanges may take electrons needed by cell

membrane for metabolism and survival. Hung went further to say 'I think that the main indicator of the effectiveness of the solution is the oxidation reduction potential, for when compared, chlorinated and electrolyzed waters, there is a differences in their oxidation-reduction potential, even though they have the same chlorine concentration. The exact mode of action of oxidation-reduction potential in killing microbes is still being investigated. Chlorine is not physically added to electrolyze water, but is produced when the electrical current passes through the water and salt mixture (Hung, 2004). The chlorine that is generated is definitely one of the components for killing microorganisms, Hung, (2000) acknowledged.

The concept and application of EW is not new. The water has been used extensively in the orient (especially Japan) for more than 25years. Its characteristics and uses have been widely documented and the Federal Department of Agriculture (FDA) has approved it for use in the United States for many years (RPA biotech, 2005). Electrolyzed water has some very interesting and highly beneficial characteristics, this are:

1. **Electrolysis restructured the water**: the process of electrolysis breaks water into smaller units, which can penetrate cells much more efficiently than normal water.

2. **The process of electrolysis change the oxidation-reduction potential:** the resulting water lacks electrons and is called oxidizing water, it has an extremely high oxidation-reduction potential an as such, it has the capacity to kill bacteria and other harmful microorganisms. Within seconds, RPA Biotech, (2005) says, 'by depriving them (microorganisms) electrons, as a topical formula, the acid electrolyzed water is a proven antibacterial, antimicrobial, and antifungal for usage on skin, foods, plants, animals, etc. It has been used as a sterilizing agent in many hospital and restaurants for years. The alkaline water has abundant electrons and is called reducing water, it has the capacity to neutralize free radicals within the body at a very efficient rate. Its antioxidant ability is unsurprised'.

3. **Electrolysis changes the water pH**: biological systems require a balanced pH to maintain homeostasis. Yet metabolic processes, stress, poor diet and undesirable environmental factors create acidic wastes, making it very difficult to maintain enough alkaline minerals in the system. As a result, most cells struggle to maintain normal body chemistry with subsequent death. These stabilized, structured and completely safe, environmentally friendly water formulations can provide benefits as never before possible (RPA Biotech, 2005).

Chapter Three

3.0.0 Materials and Methods

3.1.0 Materials

Materials for this research were soybeans (*Glycine max*), millet (*Pennisetum typhodeum*), ginger (*Zingiber officinale*), black pepper (*Pipper sp*), clove (*Euginia caryophyllata*) and cinnamon (*Xylopia aethiopica*). Sample of soybean were obtained from the seed store of the National Cereals Research Institute (NCRI), Badeggi, while ginger, millet, clove, black pepper and cinnamon were bought from the modern market Bida, Niger State. Five independent commercial producers of Soy-*kununzaki* (A, B, C, D, and E) were observed while producing the product; the weight of the ingredients used by individual producer was noted. All samples were taken aseptically and maintained at temperature ranging between 0 – 4.4°C until analyzed. For the production of electrolyzed water, sodium chloride and, distilled water were obtained from the Central Services Laboratory of the NCRI, and all other chemicals were reagent grade. The following media were used for microbiological studies, Nutrient agar (NA), for total viable bacteria counts, Eosin methylene blue agar (EMB) for coliform counts, and Sabroud dextrose agar (SDA) for yeast and fungal counts. Each medium was prepared according to the manufacturer's specification and sterilized by autoclaving at 121°C for 15 minutes.

One percent streptomycin was added to the SDA after sterilization to inhibit the growth of bacteria (Fawole and Oso, 1995).

3.1 .1 Pre-treatment of samples

Soybeans were sorted and cleaned before blanching in boiling water for about 30 minutes (Anounye, 1997), the blanched seed was then soaked in tap water for about 17-18 hours and washed in clean tap water after soaking. The millet grains were pre-treated by sorting, before soaking in tap water for about 17-18 hours and washed to remove stones, soils, glass, plastics and other foreign material found in it. The spices were cleaned and dried. The whole materials were then wet milled in an attrition mill.

3.2.0 Methods

3.2.1 Laboratory preparation of *soy-kunuzaki*

After pre-treatment, the resulting paste from wet milling was then divided into three portions. One part was left standing, while two parts was cooked by the addition of boiling water. The cooked paste was allowed to cool to about 45±2°C before mixing (2:1 v/v ratio) with the uncooked portion, which serves as a source of inoculums. The mixture was then sieved with muslin cloth in excess water followed by further

dilution with two-volume water. The filtrate (soy-*kununzaki*) is then allowed to ferment for 8-10 hours, which was then sweetened with sugar crystals to taste.

3.2.2 Preparation of electrolyzed water (EW)

Electrolyzed water used for the control of microorganisms was produced by the method described by Zhaohui, et al., (2002). It involves the electrolysis of 0.075% chlorine solution with the use of 12V battery, sodium chloride was added to distilled water and voltage applied across the anode and cathode terminals of the electrodes.

3.2.3 Hazards analysis

The hazard analysis method of Bryan, et al., (1992a) was adopted to observe soy-*kununzaki* preparation and to identify possible sources and mode of contamination, collect samples at each stage of the production process and testing them chemically, physically and microbiologically and document standard flow chart of soy-*kununzaki* processing and indicating hazards and critical control points. 200g of the raw materials were randomly sampled and the particulate mater present in the sample is counted manually and recorded as percentage physical hazards. While samples were taken at each stage of production and the ph, TTA and TSS were analyzed as shown.

3.2.4 Critical control points (CCP)

The critical control point decision tree (appendix 1) as developed by the Codex Alimentarius Committee on Food Hygiene (CODEX, 1993) and modified by Dillon and Griffith (1995) was used to identify the critical control points out of the many trivial hazards points identified during the hazard analysis (appendix 1).

3.3.0 Chemical analysis

3.3.1 Determination of pH

The pH values were measured using a referenced pH meter (Model 291 mk2 PYE UNICAM, England) after standardization with pH 4 and pH 7 buffers. Values were recorded as the meter becomes steady. The meter electrode is first dipped into a buffer solution before dipping into sample for analysis; the pH is read on the meter automatically.

3.3.2 Determination of Total Titratable Acidity (TTA)

Titratable acidity was determined using the method described by Pearson (1991). 10ml of soy-*kunuzaki* was put into a volumetric flask and three drops of phenolphthalein indicator were added and the flask thoroughly shaken. This was titrated against 0.1N NaOH to a pink color end-point. Total titratable acidity was expressed as lactic acid;

Titratable acidity = titer value (ml) x 0.009 lactic acid

3.3.3 Determination of Available Chlorine Concentration (ACC)

Available chlorine concentration (ACC) of electrolyzed water was determined by iodometric method described by Falana, et al., (2004). 4.79g of silver nitrate solution were dissolved in water and made up to one liter in volumetric flask. 0.5ml of potassium chromate solution was added as indicator into a conical flask. The sample was then titrate with silver nitrate solution with continuous swirling over a white tile until a faint permanent brick end point color is observed.

1ml of silver nitrate solution = 10ppm in a 100ml sample expressed as chlorine.

3.3.4 Determination of Total Soluble Solids (TSS)

Total soluble solids were determined in duplicate using Abbe 60 Refractometer and results expressed as degree of brix (^{o}Brix). A drop of the sample is placed on graduated face of the meter and closed; the reading was taken by viewing through the refractometer and records the high on the graduated slide. Three readings were taken and mean recorded.

3.4.0 Proximate Composition and Caloric values

Samples were analyzed using AOAC (1984) methods to determine the moisture, crude protein (N x 6.25) and crude fat. Carbohydrate content was obtained by difference, which is estimated as the difference between the total summation between of percentages of moisture, protein, fat ash and 100g (Oyenuga, 1968). The caloric values were determined by calculation using the Atweter quantification of the caloric (energy) content of food as 4.0, 4.0, and 8.9 Kcal/g from carbohydrate, protein and fat respectively (1 Kcal = 4.186kg) (Wilddowson, 1987)

3.4.1 Moisture Content Determination

Moisture was determined by the direct air-oven method of Association of Analytical Chemist (AOAC, 1984). A clean dry flat bottom silica dish was weighed and 50ml of samples pipetted in to it. The sample was evaporated on a water bath and with the aid of a forceps or tong; it was transferred into the air oven previously set at 105°C. After three hours it was removed from the oven, cooled in desiccators and reweighed. The dish was returned to the oven for another half hour, cooled in desiccators and weight again. The process was repeated until constant weigh obtained.

Moisture content (%) = A – B / C x 100

Where A = Weight of dish + sample before drying

B = Weight of dish + sample after drying

C = Original sample weight

3.4.2 Crude fat determination

Fat was extracted with solvent and determined by the Soxhlet method (AOAC, 1984). About 20ml of sample was weighed into a fat extraction chamber (thimble) of the Soxhlet apparatus and covered with cotton wool to avoid splashing during extraction. The Soxhlet extraction unit was set up and the fat extracted with petroleum ether for about 2hours. The extracted fat was then dried in the oven for about 1 hour. The fat extraction cup was cooled in desiccators and weighed, and percentage fat calculated as:

Fat (%) = weight of extraction cup – initial weight of cup / initial weight of sample

3.4.3 Crude protein determination

Crude protein was determined by the Kjedahl method (AOAC, 1984). The process involves three stages: digestion, distillation, and titration.

Digestion: about 20ml of soy-*kununzaki* sample was measured in to a 50ml Kjedahl flask, digestion tablet was then added to the flask followed by 20ml of concentrated sulphuric acid (H_2SO_4) which was poured slowly down the side of the flask while holding the flask in a slant position. The flask was then placed on a

digestion block in a fume cardboard at about 300°C until a clear solution was obtained. The flask was then made up to 100ml with distilled water after cooling to room temperature.

Distillation: about 5ml of the diluted digest of each sample was transferred into a separate Kjedahl flask and 20ml of 40% sodium hydroxide (NaOH) was added and rinsed with few drops of distilled water, the flask was then placed at the heating end of the distillation unit. 5ml of 4% boric acid solution was placed in 100ml Erlenmeyer flask and 2 – 3 drops of mixed indicator (bromo cresol green + methyl red indicators 5:1 v/v ratio) before placing at the receiving end of the distilling unit. Ammonia was distilled into the boric acid solution until the 75ml mark was reached. The boric acid distillate was then titrated with 0.1N hydrochloric acid (HCl) to a pink end point, which persists for about 15minute. Percentage protein was calculated by multiplying the percentage nitrogen with suitable factor (6.25).

% Nitrogen = A/B x C/D x 0.1 x 1/E

Where A = Volume of acid used to neutralize the distillate

B = Volume of sample taken for distillation

C = Volume made after distillation (100ml)

D = Volume of sample taken for digestion

E = Acid factor

Crude protein (%) = % Nitrogen x factor (6.25).

3.4.4 Ash Determination

Ash was determined by the method of AOAC (1984). 10ml of sample was weighed and placed in a water bath to be evaporated. It was then placed in a separate porcelain crucible (England) and its content placed in a Gallenhamp furnace and ashed at 150°C for about 10-12 hours. The samples were then removed from the furnace, cooled in a dissector and weighed. The percentage ash was calculated as:

Ash (%) = weight of crucible + ash – weight of crucible/ initial of sample

Experimental Conditions and Method of Millet and Soybeans Soaking in Electrolyzed Water

Two treatments were compared: acidic electrolyzed water and sterile water for soaking. 40g samples of soybeans and millet were soaked separately in 60ml of electrolyzed and sterile waters at ambient temperature (30±2°C) for 0.5, 1, 2, 4, 6, 8, and 10 hours each. Soaked beans and millet separated after each soaking period. The total viable bacteria count; pH, and ACC were measured. The number of microorganisms in soaks water, soybeans and millet for each soaking time was evaluated as total viable bacteria count and recorded as colony forming unit/ml (Zhaohui, et al., 2002).

3.5.0 Microbial Counts and Isolation

3.5.1 Total Viable Counts

One gram of each of the ingredients (soybeans, millet, ginger, loves, black pepper and cinnamon) and product blend at each stage of the processing was crushed or homogenized in 9ml of sterile distilled water. The mixture was then vigorously shaken to obtain a 10^{-1} food homogenate. From these serial dilutions were prepared for 10^{-2} to 10^{-6} dilutions. 10^{-6} dilution was used for plating. Total plate count was then estimated by the pour plate technique. The dilutions in the test tubes were plated in duplicates in Petri dishes, one milliliter from each from each dilution, was placed aseptically in a sterile Petri dish. Molten (15ml) nutrient agar was poured into Petri dishes. This was carefully shaken to mix the sample with agar and allowed to solidify at room temperature (30±3°C). The plates were turned over after solidification and incubated at 37°C for 48 hours. Distinct colonies formed on the agar plates counted, averaged and express as colony forming units per ml (CFU/ml).

3.5.2 Coliform Counts

One milliliter (1ml) each of the dilution was serially diluted samples were plated on EMB agar for the enumeration of coliforms. The plates were incubated at 37°C for

24-48 hours. The colonies that developed in the plates were counted and recorded as colony forming unit per milliliter of sample (CFU/ml).

3.5.3 Mold/Yeast Counts

Serially diluted samples taken from the raw materials, products at each point of processing were inoculated into Petri dishes (1ml) and molten SDA was poured and allowed to solidify before incubating at room temperature for 48 to 96 hours. The colonies that developed in the plates were counted and recorded as the colony-forming unit per milliliter or gram (CFU/ml). Series of sub culturing were made for all the counts to obtain pure culture isolates. Each isolates was grown on a nutrient agar slant and stored for next test, characterization and identification.

3.6.0. Characterization and identification of isolates

3.6.1. Direct microscopic examination

This is a differential double staining method, which forms the basis of most examinations and the preliminary identification of bacteria. A drop of sterile water was dropped in the middle of a clean glass slide. An inoculating loop was then sterilized by flaming and then cooled before touching the bacterial colony with the loop, after which the bacterial cells were rubbed in the water on the slide and spread into a thin smear along the slide. The smear was then air-dry and then the reverse

side of the slide was passed quickly three times over a flame in order to fix the bacteria. The slide was then allowed to dry before staining. In this method, bacteria are first stained with crystal violet and then treated with iodine solution. The bacterial smear is next treated with alcohol, which entirely removes the crystal violet in the case of gram-negative bacteria. Dilute safranin may be used as a counter stain. This method divides bacteria into to two classes: gram positive, these do not decolorize with the application of alcohol, thus appearing purple. Eighten to twenty-four hours (18-24hrs) old colonies were used for gram staining.

3.6 2 Biochemical Tests

Biochemical tests (Collins and Lyne, 1984) done for further identification of the isolates include:

3.6.3 Test for Motility

Some bacteria possess flagella and therefore, can move. Such motility can be demonstrated by the hanging-drop technique (Fawole and Oso, 1995). A little liquid paraffin was placed round the edge of the depression of cavity slide. A loopful of colony was transferred to the center of a clean dry cover slip placed on a flat sheet of paper. The cavity slide was then inverted over the cover slip such that the culture drop is in the center of the of the slide depression. Press the slide down carefully but firmly so that the liquid paraffin seals the cover the slip in position. The slide is then

quickly and carefully inverted and the culture drop now appears 'hanging'. The slide is then observed under microscope.

3.6.4 Catalase Test

Most aerobic microorganisms are capable of producing the enzyme catalase although to different extends. This physiological reaction is used in characterization and identification of microorganisms. An emulsion of isolate was made on a clean slide with normal saline. A drop of hydrogenperoxide (3% H_2O_2) was added and the slide observed, effervescence caused by the liberation of oxygen as a gas bubble indicate the production of catalase enzyme by the bacterium, the result was recorded positive, but where no such bubbles, the result is reported as negative (Fawole and Oso, 1995).

3.6.5 Indole Production

Some microorganisms are capable of hydrolyzing the amino acid tryptophan and one of the end products is indole. The ability of a microbe to carry out this reaction can be used for biochemical characterization. The tryptone in the culture media supplies the tryptophan. The isolated colonies were incubated at 37°C for 24 hours in peptone broth. After the incubation period, 2ml of Kovac's reagent (p-dimethyl-aminobenaldehydes) was added and shaken gently. The test tubes were then returned to the test tube rack and allowed to stand for about 20minutes before

taking record. A red color at the reagent layer was recorded as indole positive (Fawole and Oso, 1995).

3.6.6 Coagulase Test

This test is used to differentiate *Staphylococcus aureus (*coagulase positive) from *S. epidermidis* and *S. saprophyticus* (coagulase negative). The enzyme coagulase clots plasma by converting fibrinogen to fibrin. A drop of saline was placed on a clean glass slide and a loopful of test organisms was emulsified. A drop of plasma was added to the emulsion and mixed before observing. Clumping of the organisms within 10 seconds (Collins and Lyne, 1984) indicate positive result.

3.6.7 Sugar utilization test

Lactose, glucose, fructose and maltose etc, were used to perform the test as described by Collins and Lyne (1984). Two percent (2%) each of the sugar were prepared by dissolving 2g of sugar in 100ml of 2.5% peptone water. The solution was then dispensed into test tubes and sterilized, then cooled and inoculated with the test organisms and incubated at 37°C for 18 to 24 hours before observation.

3.6.8 Identification of Mould and Yeast

Identification of fungi depends largely on morphological characteristics such as the type and arrangement of spores produced, as well as the mycelia type. The isolates

were assigned to probable identity using Taxonomic description in Samson and Reenen-Hockstra (1988).

3.6.9 Statistical Analysis

Data collected were subjected to analysis of variance (two way ANOVA). This was used to evaluate variation in terms of changes in microbial count and chemical parameters at each stage and their interactions. Probability level was maintained at 0.05 (confidence limit) (Gomez and Gomez, 1984). While Duncan's Multiple Range Test (DMRT) was used to test significance within the mean of the treatments (Ignatius, 1986). While process capability index was analyzed using statistical quality control formulas described by Anonymous (2005).

CHAPTER FOUR

4.1.0 Results

4.2.0 Identification of Hazards and Critical Control Points

Table 2 shows details of the hazard analysis of Soy-*kununzaki* production and its critical control points (CCPs). Stones, pieces of metals, straws, and wood, plastics, and broken glass pieces were the main physical hazards of concerned identified. There were also material, time ad energy looses recorded at few other points in the process operation. While figure 7 shows details of the flow chart followed by all the processor in the production of Soy-*kununzaki,* it was monitored and the critical control points identified. The critical control point identified were washing operation, steeping of raw materials, milling operation, sieving and holding of paste for fermentation, and packaging.

Table 2: Hazards identified in the processing of Soy-kununzaki and its control points (CCP)

Hazards*	Amount	Operation	CCP
Physical			
Stones	4.7g	ST, CL	ST
Plastics	2.5g	,,	ST
Metals	3.9g	,,	ST
Soils	14.0g	,,	ST
Weed seeds	11.2g	,,	ST
Broken grain	3.1g	,,	SK
Temp. variation	-	SK, MS, STD	ST
Biological			
Animal wastes	5.3g	ST, CL	ST, CL
Insects	4.3g	,,	SK
Microorganisms	-	all operations	CL MS, STD
Microbial growth	-	all operation	SK, STD
Chemicals			
Residue from detergents	ob	Cleaning	CL
Absorption from water	ob	SK	SK
Absorption from air	ob	SK, STD	STD
Particulate from environment	ob	SK, STD, ML	STD
Others			
Waste to the environment	ob	SK, ML, SV	SV, ML
Material losses	ob	ML, SV	SV, ML
Time losses	ob	ST, CL, STD	STD
Energy losses	ob	ML, MS	ML, MS

*Hazards are physical, chemical and biological agents that are reasonably likely to cause illness, injury or loss in the absence of control.

SK=Soaking, **ML**=Milling, **SV**=Sieving, **STD**=Standing, **MS**=Mixing, and **CL**=Cleaning, **ST**=Sorting.

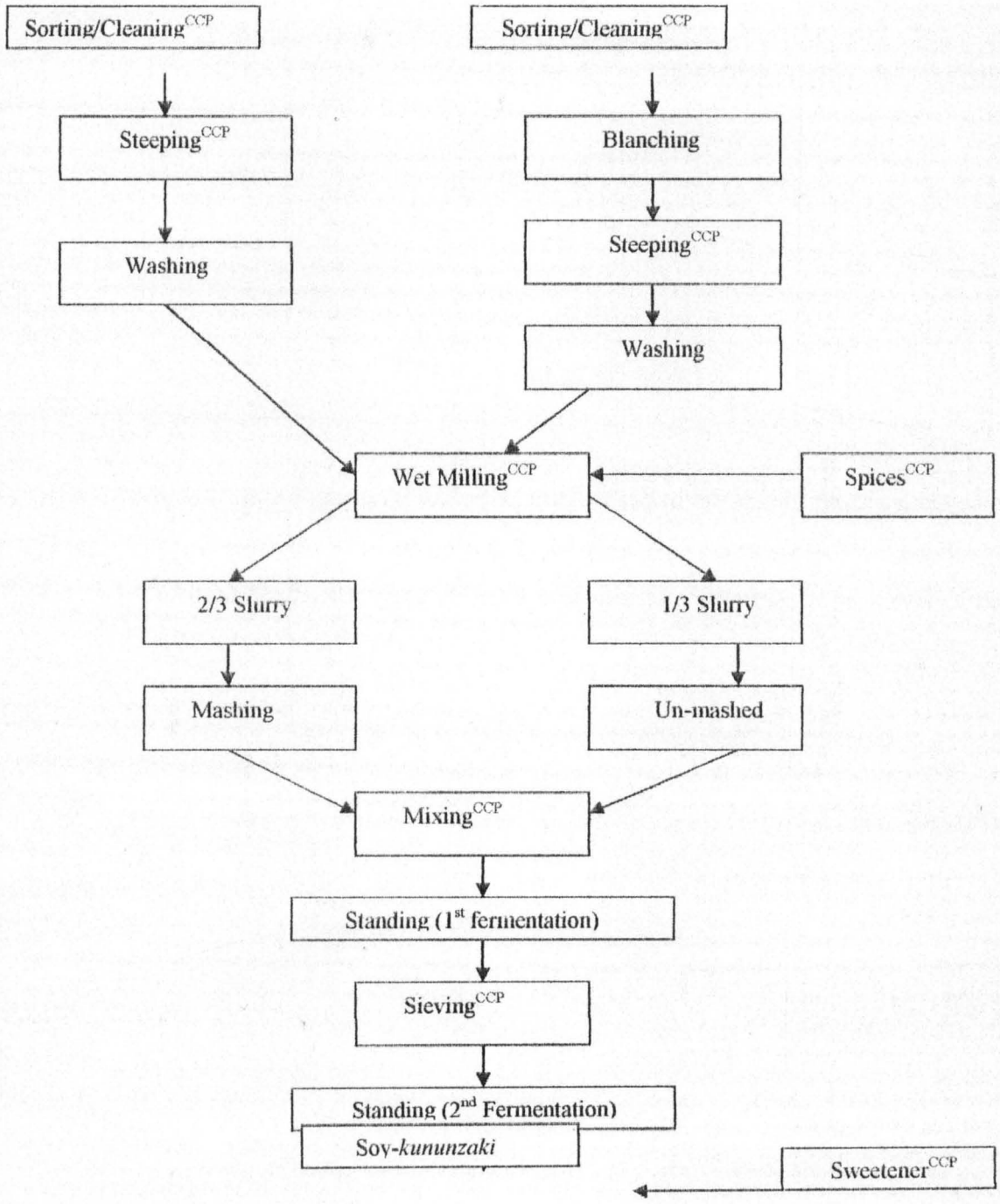

Figure 6 Soy-*kununzaki* production chart showing critical control points (CCP)

Table 3: Change in pH during soy-*kununzaki* fermentation (production).

Fermentation Time (hours)	Change in pH*
0	6.314^{a}
2	6.122^{a}
4	5.538^{b}
6	4.230^{c}
8	4.038^{cd}
10	3.804^{de}
12	3.634^{e}

*Value of three recorded as mean. Mean of the same column followed by different letter differs significantly according to Duncan's Multiple Range Test at 5% level of probability.

Table 4: Change in Total Soluble Solids in Soy-*kununzaki* during fermentation (Production).

Fermentation Time (hour)	Total Soluble Solids (°Brix)*
0	14.140^{a}
2	13.388^{ab}
4	12.592^{bc}
6	11.820^{c}
8	9.646^{d}
10	8.812^{de}
12	8.220^{e}

*Value of three recorded as mean. Mean of the same column followed by different letter differs significantly according to Duncan's Multiple Range Test at 5% level of probability.

Table 5: Change in Total Titratable Acidity (TTA) in Soy-*kununzaki* during fermentation (production)

Fermentation Time (hour)	Total Titratable Acidity*
0	0.180^{g}
2	0.274^{f}
4	0.382^{e}
6	0.482^{d}
8	0.604^{c}
10	0.706^{b}
12	0.820^{a}

*Value of three recorded as mean. Mean of the same column followed by different letter differs significantly according to Duncan's Multiple Range Test at 5% level of probability.

Table 6: Change in chemical attributes of Soy-*kununzaki* among five producers during its production.

Producer	Chemical attributes		
	PH*	TTA**	TSS**
M1	4.830	0.384c	12.719a
M2	4.860	0.501b	10.770c
M3	4.933	0.506ab	9.940c
M4	4.780	0.551a	11.089bc
M5	4.648	0.520ab	12.239ab

*Value of three recorded as mean. **Mean of the same column followed by different letter differs significantly according to Duncan's Multiple Range Test at 5% level of probability.

Table 7 Number of microorganisms isolated on the raw materials used in the production of Soy-*kununzaki*

Raw Material	TVC (CFU/ml)	CFC (CFU/ml)	MYC (CFU/ml)
Soybeans	$1.26x10^3$	$1.58x10^4$	$<1.00x10^1$
Millet	$1.68x10^3$	$2.98x10^3$	$6.20x10^3$
Ginger	$4.18x10^4$	$<1.00x10^1$	$2.21x10^3$
Black pepper	$1.43x10^3$	$2.72x10^4$	$1.10x10^3$
Cinnamon	$1.01x10^3$	$4.42x10^3$	$<1.00x10^1$
Clove	$<1.00x10^1$	$5.36x10^4$	$<1.00x10^1$

Values of three counts recorded as mean.

TVC = Total Viable Count

CFC = Coliform Count. **MYC** = Mold and Yeast Count.

Table 8: Change in the number of microorganisms counted at different stages of Soy-*kununzaki* production.

Production Stages	TVC+ (CFU/ml)	CFC+ (CFU/ml)	MYC+ (CFU/ml)
Steeping (SB)	$4.26x10^4$	$1.85x10^3$	$3.27x10^4$
Steeping (ML)	$4.89x10^4$	$3.18x10^3$	$3.11x10^3$
Paste	$6.48x10^4$	$4.02x10^3$	$5.12x10^3$
Cooked +uncooked	$2.26x10^2$	$2.89x10^3$	$5.00x10^3$
Filtrate	$3.86x10^3$	$2.41x10^2$	$2.13x10^3$
Filtrate	$4.92x10^4$	$1.10x10^2$	$<1.00x10^1$
Filtrate	$5.64x10^4$	$1.18x10^2$	$<1.00x10^1$
Filtrate (final product)	$6.13x10^5$	$<1.00x10^1$	$<1.00x10^1$

+ = Value of three counts recorded as mean.

- **TVC** = Total Viable Count
- **CFC** = Coliform count
- **MYC** = Mold and Yeast Counts.

4.3.0 Changes in Chemical Attributes of Soy-*kununzaki* During Production

4.3.1 Change in pH

Figure 7 shows the change in the pH during production. There was a drastic change in the pH from weak acid reaction at the beginning (steeping) of the production process to a relatively strong acidic reaction at the end (filtrate) of the production. The pH ranged between 6.02 to 3.16, 6.31 to 3.61, 5.98 to 3.82, 6.87 to 3.66, 6.06 to 3.68 and 6.05 to 3.22 for processors A, B, C, D, and E respectively. The average pH ranged between 6.22 to 3.53 and the highest value recorded was 6.87 for processor C and the lowest value recorded at the end of the production process was 3.16 from processor A. Statistical analysis of the data shows that there was no significant difference ($p \leq 0.05$) between the values recorded from the five processors (Appendix 2)

4.3.2 Change in Total Titratable Acidity

Figure 9 shows the changes in total titratable acidity in soy-*kununzaki* during production among the five sampled producers. The value increases even as pH decrease from an average of 0.053 to 0.099 with the highest value of 0 .104 recorded from processor D, and the lowest of 0.016 from the processors B and C. Among the processors, the increase was from 0.016 to 0.93, 0.016 to 0.102, 0.078

to 0.094, 0.102 to 0.173, 0.016 to 0.104 and 0.017 to 0.096 for A, B, C, D, and E respectively. Statistical analysis shows that there was a significant difference ($p \leq 0.05$) in the change in total titratable acidity among the five processors (Appendix 2).

4.3.3. Change in Total Soluble Solids

The percentage soluble sugar (^{o}Brix) gradually decreases from an average of 8.05 at the beginning of the operation to 6.68 at the end of the process. The values changes from 9.00 to 7.00 in samples from processor A, while 8.10 to 7.00, 9.05 to 6.67, 7.67 to 6.38, 8.00 to 6.65 for processors B, C, D, and E respectively. There was a relative significant difference observed ($p \leq 0.05$) among the soluble sugar values from the different processors (Appendix 2).

4.4.0 Number of Microorganisms counted on Raw Materials used for the Production of Soy-*kununzaki.*

Table 3 shows the numbers of microorganisms isolated on the raw materials used for the preparation of Soy-*kununzaki*. Total viable bacteria count ranged between 4.18×10^4 to less than 1.00×10^1 CFU/ml. The count was high in ginger with 4.18×10^4 CFU/ml followed by Millet 1.68×10^3 CFU/ml. The result of the Coliform count was high in Cloves with a count of 5.36×10^4CFU/ml and Cinnamon 4.42×10^3 CFU/ml. The least count was recorded in Ginger (less than 1.00×10^1CFU/ml).

Mold and Yeast counts were generally low, with the exception of Millet, which has a record of $6.20x10^3$CFU/ml and $2.21x10^3$CFU/ml in Ginger.

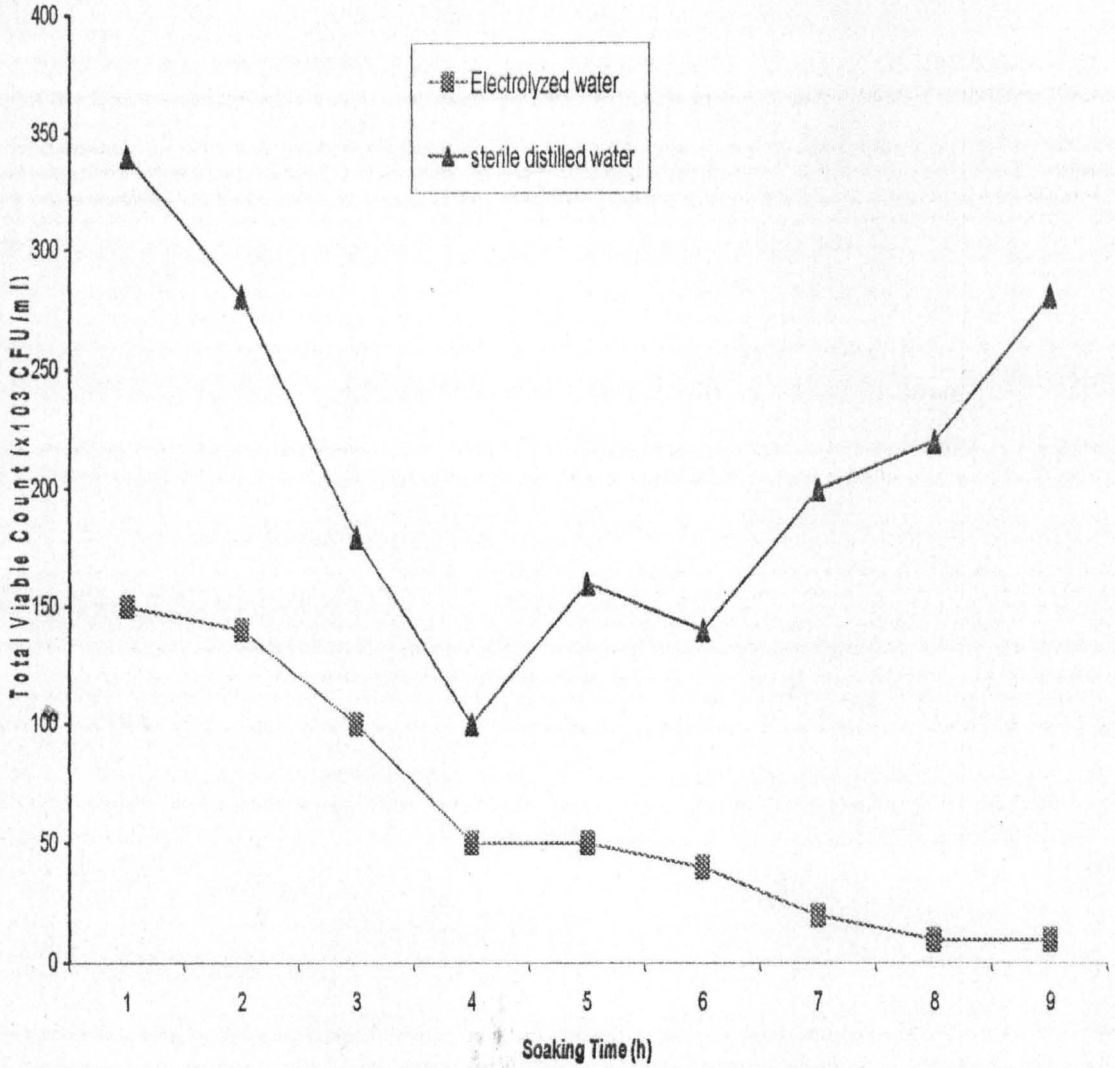

Figure 10 Changes over time of Viable Bacteria Count in soaked Soybeans

4.4.1. Changes in the Number of Organisms Isolated at Different Stages of Soy-*kununzaki* Production.

The number of viable bacteria count at the beginning of the production process is shown table 4. The count was relatively high, and steadily increases from $4.26x10^4$CFU/ml at soaking stage to about $6.48x10^4$ CFU/ml at the paste level. It then drastically falls to about $2.26x10^2$CFU/ml when the cooked and the uncooked were mixed. It then starts to increase gradually to $6.13x10^5$ in the final products. Coliform count increases from $1.86x10^3$CFU/ml at the beginning of the soaking process of Soybeans, and $3.18x10^3$CFU/ml in millet soak water to $4.02x10^3$CFU/ml at the paste level, before falling to $2.89x10^3$ CFU/ml when the cooked and uncooked were mixed and gradually decreases to less than $1.00x10^1$ CFU/ml at the end of the process. Result of yeast and mold count indicated a gradual increase from $4.11x10^3$ CFU/ml in the millet soak water to less than $1.00x10^1$ CFU/ml at the end of the process.

Table 9: Bacteria isolated from raw materials used in the preparation of soy-*kununzaki*

Raw materials	Microorganisms
Soybean	*Staphylococcus spp*
	Bacillus spp
Millet	*Shegilla spp*
	Lactobacillus spp
Ginger	*Klebsiela spp*
	Bacillus spp
	Staphylococcus spp
Black pepper	*Bacillus spp*
	Bacillus spp
Cinnamon	*Klebsiela spp*
Cloves	*Micrococcus spp*
	Staphylococcus spp

4.5.0 Isolation and Characterization of Isolates from Raw Materials used for Soy-*kununzaki* Production.

The bacteria isolated and identified from the raw materials used are shown on table 5. Species of *Bacillus, Staphylococcus, Micrococcus, Klebsiela, Shegella,* and *Lactobacillus* were identified. While the species of mold identified were *Aspergillus, Mucor, Rhizopus nigrican, Penicillium,* and *Trichophyton. Bacillus* species were the dominant organisms isolated from Ginger and Soybeans, while *Aspergillus* appeared frequently in almost all the raw materials. *Staphylococcus spp* and *Bacillus spp* were isolated from Soybeans, while *Shegilla spp* and *Lactobacillus* were isolated from Millet. *Klebsiela spp, Bacillus spp,* and *Staphylococcus spp* were detected on the ginger, and *Bacillus spp* was the only organism isolated on the black pepper. *Klebsiela spp* was also recorded on Cinnamon and *Micrococcus* and *Staphylococcus spp* on Cloves.

Table 10: Fungi isolated from raw materials used in the production of Soy-*kununzaki*

Raw materials	Organisms
Millet	*Aspergillus niger*
	Mucor spp
Soybean	*Aspergillus niger*
Clove	*Trichophyton spp*
Ginger	*Penicillum spp*
Cinnamon	*Rhizopus spp*

Table 11: Yeast isolated from soy-*kununzaki* during production

Isolates code	organism
Sk-12	*Trichosporon spp*
Sk-13	*Torulopsis spp*
Sk-22	*Saccharomyces cerevisiae*
Sk-32	*Candida spp*
Sk-11	*Candida spp*
Mm-1	*Cryptococcus neoformance*
Mm-31	*Candida spp*
Fl-43	*Candida spp*
Fl-21	*Torulopsis spp*

Table 12: Bacteria isolated from soy-*kununzaki* during production

Isolate code	Organism
Bt 00	*Bacillus spp*
Bt 43	*Bacillus spp*
Bt 54	*Lactobacillus spp*
Bt 12	*Klebsiella spp*
Tr 32	*Corynebacterium spp*
Re 21	*Staphylococcus spp*
We 33	*C. haemolyticum*
Wq 51	*Listeria monocytogenes*
Rf 34	*Micrococcus*

Table 13: Proximate Composition of Soy-*kununzaki* Produced under Laboratory and Manufacturer's Condition

Quality attributes	Laboratory sample	Processor A sample
Crude protein	2.21±0.10	2.11±0.04
Ash (%)	0.12±0.13	0.11±0.06
Fat (%)	5.43±0.05	6.34±0.12
Moisture (%)	84.21±0.4	86.65±0.12
Carbohydrate (%)	7.03±0.11	4.99±0.14
Caloric value (Kcal/g)	487.27±0.06	564.26±0.18

Mean + standard deviation of three determinations

(1Kcal = 4.186KJ)

4.5.1 Proximate and Caloric value of Soy-*kununzaki*

The proximate composition of Soy-*kununzaki* from one of the processors and that of the laboratory prepared samples are presented on the table 10. The result indicated that there was no much difference in the value of protein content as the values were just slightly above 2 (2.21% and 2.11%) for laboratory and field production respectively. The fat content was 5.43% and 6.34%, while energy values were 487.27Kcal/g and 564.26Kcal/g respectively.

4.6.0 Sterilizing effects of electrolyzed water

The changes in the viable bacteria count for the period of soaking is shown on figure 12. Approximately $1.26x10^3$ CFU/ml and $1.68x10^3$ CFU/ml of microorganisms were detected in the original soybeans and millet (table 6). The count decreases steadily within the first few hours, and falls to a negligible value at the end of the soaking time. The number of bacteria in soybeans soaked in sterilized water decrease initially but then increases after 4 hours of soaking. The changes in viable counts in soybeans and millet soak water are shown on figure 13. The results indicates that during the first four hours of soaking microbial bacterial growth was low and steadily increase sharply, in both the soak water similar trend was observed.

Table 14: Change in pH of soaking water (EW) during steeping of soybean and millet

Time (Hour)	PH	
	Soybeans	millet
0	2.6	2.0
2	3.0	2.8
4	4.3	3.6
6	4.3	4.0
8	4.9	4.3
10	5.6	5.8
12	6.8	6.5

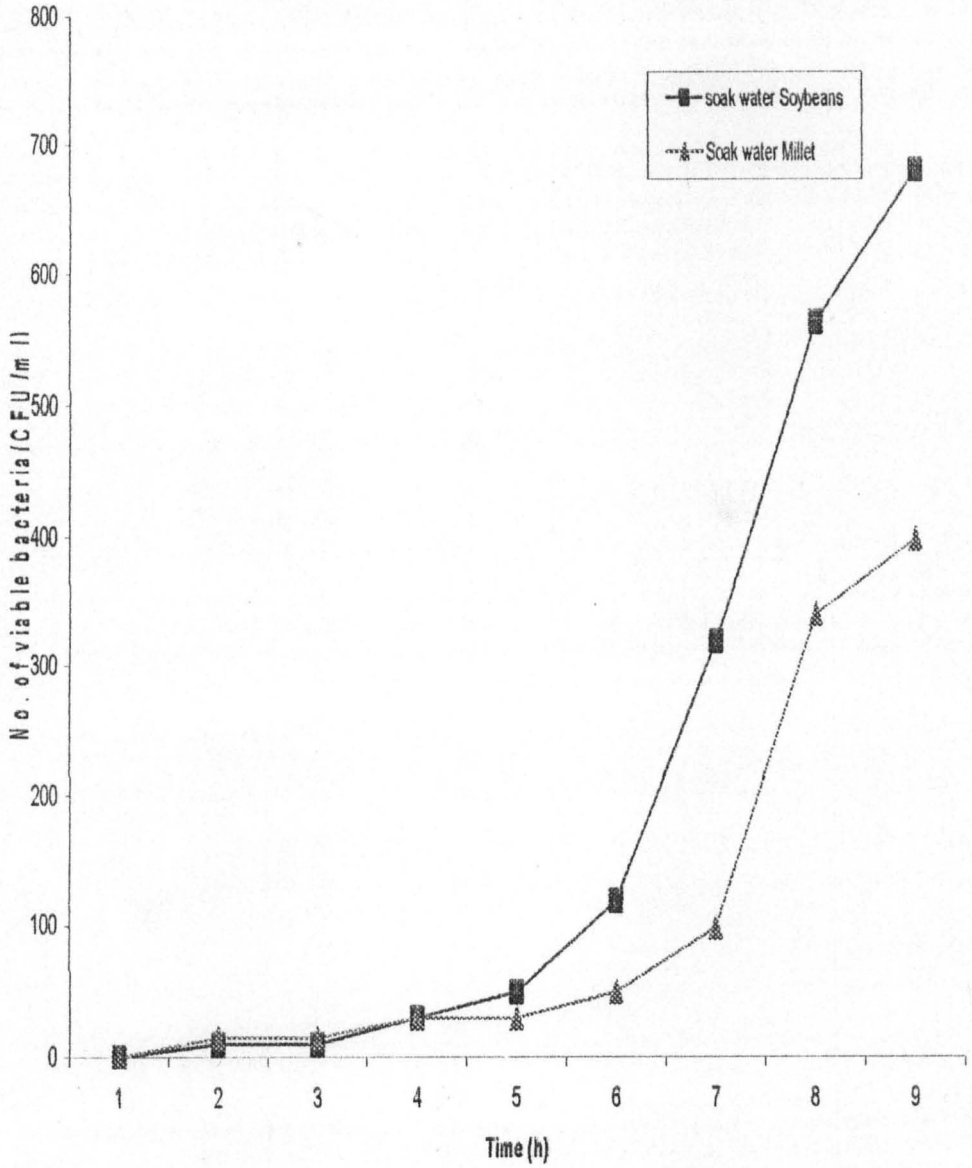

Figure 11 Changes in Viable Bacteria Counts over time in Soybean and Millet soak water.

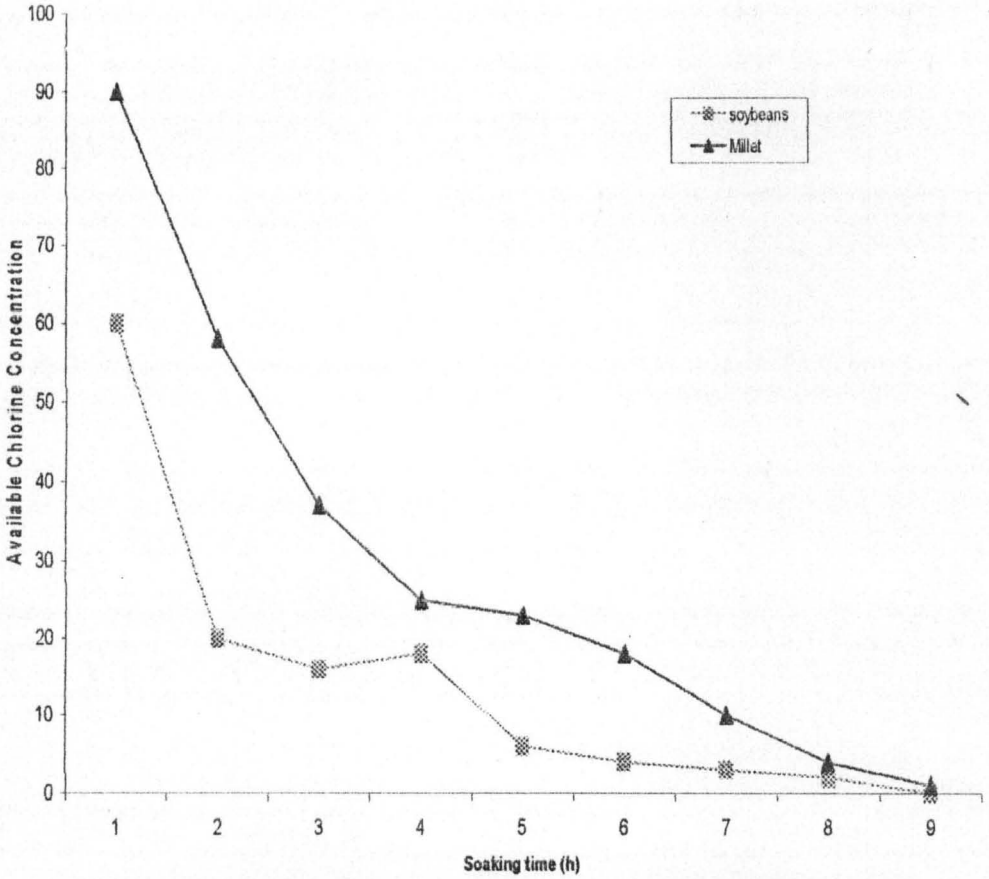

Figure 12 Changes in available chlorine concentration in soaking water after soaking Soybean and Millet for 10hours

4.6.1Change in Available Chlorine Concentration (ACC) and pH during Soybean and Millet soaking

Figure 12 shows the change in available chlorine concentration during the soaking time of both millet and soybeans. The available chlorine concentration decrease rapidly at the first 2hours of soaking, and then decreased gradually until the termination of the experiment. After about 12 hours of soaking, the residual chlorine concentration of the electrolyzed water was about 5ppm.

4.7.0 Process Capability Test

For the test of the process capability, the process of the laboratory preparation steps are used and the limits set by WHO are used as upper and lower specifications. The upper and lower specifications are 10^3CFU/ml and 10^4CFU/ml. The observed process mean of microbial count was 10^2 CFU/ml, and the standard deviation, s is 0.988. From this we can obtain our process capability (Cp).

Cp = Process capability index

USL = Upper specification limit

LSL = Lower specification limit

S = Standard deviation

The upper and lower microbiological specification of raw agricultural products are 10^4 and 10^5 respectively, this specification also applies to finished cereal products like *kunu, akamu,* custard, *pito etc.*

Therefore, for bacterial counts,

$$Cp = USL - LSL / 6s,$$

$$= 10^4\text{-}10^3 / 6s$$

$$= 10^4\text{-}10^3 / 6x0.988$$

$$= 10/5.928 = \mathbf{1.687}.$$

And for coliform count,

$$Cp = USL - LSL/6s$$

$$10^4 - 10^3 / 6x1.02$$

$$10/6.12$$

$$= \mathbf{1.634}$$

CHAPTER FIVE

5.0.0DISCUSIONS

The physical hazards identified are presented in table 2. They are stones, pieces of metals, glasses, and soil, seeds of the plants, and plastics. There was also mechanical, time, and material losses recorded at different points in the process. The presence of these hazards in excess of the acceptable limits is considered of high risk and may cause cuts, injury, bleeding, infections, trauma etc. Mechanical damage to the milling machine and sieving cloths may also occur, and may lead to time and material loss.

The significant hazards identified in the Soybean during sorting and washing were mechanical damage to the grain itself, high microbial load, and the presences of poisonous and dead insects. When damage is done to the Soybeans FAO (1977) and IITA (1987) it disrupts the cell of the beans and this triggers a chemical reaction initiated by isoenzyme of lipoxygenase present in the cells of the beans. These isoenzymes oxygenate poly-unsaturated fatty acids containing cis-1, 4-pentadiene double bound located between the carbon 6 and 10 from the methyl end of the fatty acid. In the presence of linoleic acid the isoenzyme produces hydro-peroxide that may be responsible for the beany flavor of the product. The dead

insects may also impact off flavor and taste to the product if not removed. The injury that may be sustained as a result of the presences of these physical hazards in excess of the acceptable limits may require surgery to remove. Most of the physical hazards are removed during sorting and cleaning process that is given to material before going into the processing line. Therefore, HACCP approach is effective in preventive, elimination or reduction of these hazards to acceptable levels, this also agrees with the report of Foster, (1989) and Buchaman, (1990) who believed that sorting and cleaning are critical in the preparation of cereal-based products. Microbiological hazards identified include bacteria, mold and yeast. These organisms were associated with the raw materials used (tables 9, 10, 11, and 12). ICMSF (1986), Sanni, (1997) and FDA (2005) reported that many of these organisms are pathogenic, and occur naturally in the environment where foods are grown and processed.

The aim of washing is to clean the surface of the raw materials, to remove particulate materials and to reduce microbial load on them. Loss of control at this point could lead to the identified hazards in excess of acceptable limits. Unfortunately, the step that follows was not designed to eliminate or reduce the likely hood of the occurrence and extent of this hazard to food and consumer safety. Therefore, washing was judged as critical control point.

During the soaking process that follows, washing of the soaked soybeans and millet may reduce the identified hazards, but the soaking time of 8 to 10 hours allows for the rapid growth of the microbes present (Table 8). Also, the dissolved solids from millet and soybeans serve as sources of nutrient for the propagation of microbes. Loss of control at this stage may lead to the identified hazards in amount greater than the acceptable level of 10^4 - 10^3CFU/ml in beverage. But milling which is the next step in the process was not designed to eliminate or reduce the microbial hazards to acceptable levels; hence, soaking is considered a critical control point.

Surprisingly, in all the processors monitored, none of them was seen washing their machine thoroughly before milling. Tomatoes, onion, beans, sorghum, and pepper were milled in the machine the previous day. These materials have different microbiology with soybeans, millet and spices used for Soy-*kununzaki* preparation, therefore, re-contamination, change in color and taste of the product may occur. Unfortunately, the next step was not designed to eliminate or reduce the hazards. Therefore, milling is considered a critical control point. The most important hazards of health risk identified during the sieving operation are the pieces of metals. This was learnt to be as a result of new disks used during milling. These fine metal pieces are of great health risk to the consumer. The presence of metal

pieces in wet milled products agrees with the finding of Nilson et al.,(1979) and Karl, (1987). The water used to dilute the paste before sieving may probably serve as source microbial recontamination, since the next step cannot eliminate or reduce the hazards to acceptable levels, sieving is considered critical control point. Mixed pastes were kept at room temperature in an open container for 2-4 hours before sieving, and for 7-10 hour for fermentation. Holding foods at warm ambient temperature for 3 to 6 hours present high safety risk; the risk increases substantially with every hour of holding. Jideani, et al (2001) reported that daytime temperature of less than 40^0C at mid day hours, were conducive for promoting microbial growth. The beverage is allowed to stand for over 8 hours to ferment, the taste and flavor change during this period and preferred by most consumers, but holding the beverage at that temperature for that long allows for microbial proliferation (Table 8 and 10) and possible recontamination from the environment. Unfortunately no subsequent treatment was given to the beverage after this step to reduce or eliminate the hazards; hence, holding is considered critical control point. Soy-*kununzaki* are distributed with minimal packaging, they are mainly packaged in cellophane bag or plastic bottles. The processors were seen blowing the bag with their mouths; this is high-risk point as recontaminations with pathogenic

microorganism of human origin are high. Packaging therefore, is considered critical control point.

Some of the microorganisms isolated (*Bacillus spp, Listeria spp, Staphylococus)* from the raw materials (Tables 9 and 10) and the beverage (Tables 7 and 12) are of public health importance. The isolation of these organisms is in line with the work of Onuorah, et al., (2001) who reported that *Listeria spp, Staphylococcus spp* and *Bacillus spp* are bacteria of significance in food borne diseases and they cause some of the known bacterial food borne illness. *Listeria monocytogenes* has since become recognized as an important food borne pathogens. According to Lovett and Twedt (1998), Specticemia is the most common *listeric* manifestation in adults. The isolation of these organisms in Soy-kununzaki agrees with the report of Anounye, (1997), and Akoma, et al. (2002). Forms of Listeriosis involving the central nervous system include meningitis, encephalitis and abscesses (Lovett and Twedt, 1998). *Staphylococcus* food intoxication is one of the most common food-borne illnesses giving rise to nausea, vomiting, abdominal cramping, prostration and diarrhea. Patel, et al., (1976), Mason (1979), Onuarah, et al., (1987) and Efiuvweuwere and Akoma (1995 and 1997) all reported the contamination of raw materials used for *kununzaki* production by *Staphylococcus.* The toxins produced by *Staphylococcus spp* are somewhat heat resistant, and therefore it is possible to

have *Staphylococcus* food poisoning, having resisted the heat treatment during blanching and addition of hot water. The most important sources of *Staphylococcus* are the human. These buttress the report of WHO (2005), that about 40% of normal human adults harbors these organisms in the nose and throat, hence the finger tips of human are often contaminated with these bacteria. Consequently, when contaminated foods are held for several hours at temperature well above 6.6^0 C the *Staphylococci* will grow and produce toxins. Therefore, personal sanitation by food processor and temperature at which the product is to be kept are considered critical. Some species of *Staphylococcus* are also considered lipolytic organisms. They are therefore, capable of causing hydrolytic and oxidative deterioration of the fat contained in the beverage. *Bacillus spp* are also proteolytic (Byerklie, 1992) and therefore produce variety of odor and flavor defects in the final product if not properly taken care of Faparusi, et al., (1983) have also reported the presence of *Sacchaomyces cerevisiae*, *Candida spp*, and *Aspergillus spp* isolated on cereal grains. The difference in the types isolated at different stages of production by the different producers may likely be due to different environmental conditions under which the beverage were prepared and the type of organisms each individual producer might be harboring. *Aspergillus niger* and *Aspergilus flavus* were the most isolated mold through out the

production process, but *Sacharomyces cerevisiae* dominate at the late stage of the process. Anon (1980) reported the same trend; this may likely be as a result of increased acidity. The increased acidity may provide some assurance of microbiological safety of the product, as most of the organisms count reduced with increased acidity. But this may be doubtful since very high total viable population exceeding maximum levels recommended for beverage is not accepted. The number of bacteria isolates at different stages from different producer are higher than either the yeast or mold isolated, this could be due to environmental factors and the extent of contamination together with the fact that bacteria grow faster than yeast and fungi. Most of the bacteria isolated do not have any beneficial functions in beverage production, except for some *Bacillus* species involved in fermentation. Jideani and Osume (2001) reported that some *Bacillus spp* are involved in fermentation in some local alcoholic beverages. The yeasts are involved in the fermentation process. Isolates such as *Lactobacillus spp, Candida* and *Aspergillus* apart from being contaminants, are reported to be involved in most fermentation process such as converting mash to wort. *Torulopsis* species are associated with food spoilage, being common contaminants in breweries. Although *Lactobacillus* spp. and yeast are not pathogenic, they may cause significant quality and economic losses, making there control desirable.

The crude protein content in the final product forms one of the bases for this work. It was observed that the percentage of crude protein in the beverage was higher than the values recorded in other *Kununzaki* produced with only cereal as the principal raw material. The crude fat content is relatively high; this may be attributed to the soybeans used, since it is an oil seed. This may affect the storage ability of the product. The energy values falls within the reference value of 450 – 600Kcal/100g for humans of all age groups as reported by Abdullahi, 2001. The energy content of the beverage (Table 13) is also within the limits recommended for infants between 4 to 11 months of age by the World Health Organization (FAO/WHO, 1985).

It is often required to compare the out put of process optimization within the process specifications recommended by regulatory agencies, and make statement about how well the process meets our objectives (Stevenson, 1990 and Price et al., 1993). Statistically we assume that the results follow normality. The process capability index (Cp), as calculated was approximately 1.7 for bacterial count and 1.6 for coliform. Anonymous, (2005) reported that for a process to be considered capable, the Cp would have to be at least 1.0 this means that the process HACCP is capable of controlling hazards in soy-*kununzaki* preparation if carefully implemented. The resulting change in acidity toward alkalinity during steeping

might be due to a reaction between sodium hydroxide with carbon dioxide from the atmosphere and/or from soybean and millet seeds respiration. These changes in pH buttress the finding of Koseki and Itoh, (2000a). It is also noted that some materials of the soaked soybeans and millet dissolve in the soak water, some of this materials might have brought about the decrease in acidity. The antimicrobial activity of electrolyzed water has been attributed to the relationship between pH, oxidation-reduction potential (ORP) and available chlorine concentration (Koseki and Itoh, 2000b). Studies by Koseki and Itoh, (2000a, 2000b) and Zhaohui, et al., (2002) have also shown that the main factor in the elimination of microorganism by electrolyzed water is available chlorine concentration, the hydroxyl free radical produced from hypochlorous acid is available chlorine which acts on microorganisms. The gradual decrease in the value of available chlorine concentration (figure 12) may be as a result of the reaction of chlorine with the organic matters in these materials. The hypochlorous acid around available chlorine primarily acts as a bactericidal against (Kijima et al., 1997). While hypochlorous ion has less bactericidal action as a result of its negative charge, which makes it difficult to penetrate the cell membrane.

5.1.0 Conclusion

This study has drawn attention to the fact that hazard analysis critical control point approach in quality management can be used in the preparation of the beverage. The hazards identified are of great concern, and therefore their identification and documentation will go a long way in contributing to scholarly literature on this product. The critical control points identified will be a point for all processors of this product and similar to take maximum care so that safe food could be produce, which will increase consumer confidence and in turn more patronage. The presence of spoilage and contamination organisms is an indication that the produce is not produce under good hygiene practices and poses a serious health risk to its direct consumers. It was shown local processors could use the electrolyzed water as a means microorganism control in raw materials used for food preparation. The HACCP approach therefore, has been shown to identify areas of concern where failure has not yet been experienced, making it particularly useful for new operation.

5.2.0 Recommendations

Based on the results of the study the following recommendations are made:

1. Processors of this beverage need to be trained in good manufacturing practices and the need to properly clean raw materials intended for soy-*kununzaki*

production. Because educating and training of all personnel are critical to the success and effective application of HACCP. The training plan should be specific to the establishment and product's operations rather than developing expertise for broad application.

2. There is the need to conduct thorough research in to the possible chemical hazards associated with soy-*kununzaki,* as this work only analyses physical and microbiological hazards in details.
3. An effective national food safety program need to be put in place by the national government, and supervisory and enforcement left to local and states agencies.
4. Because the informal food sector is composed of large, small, chain and independent units, specific HACCP plans for the products produced and sold as ready-to-eat foods should be produced and made compulsory for the registration of the product by NAFDAC or any other regulatory body.
5. Training reinforcement is also needed for continued motivation of the local food processors. It may come in the form of video/TV training program. It may also be in a form of bold prints on the wall such as "HAND WASHING PAYS DIVIDENDS", "CONSUMERS HEALTH IS IN YOUR HANDS". Or in the form of workstation reminder such as pictorials on hazards associated with each step in the food processing operation.

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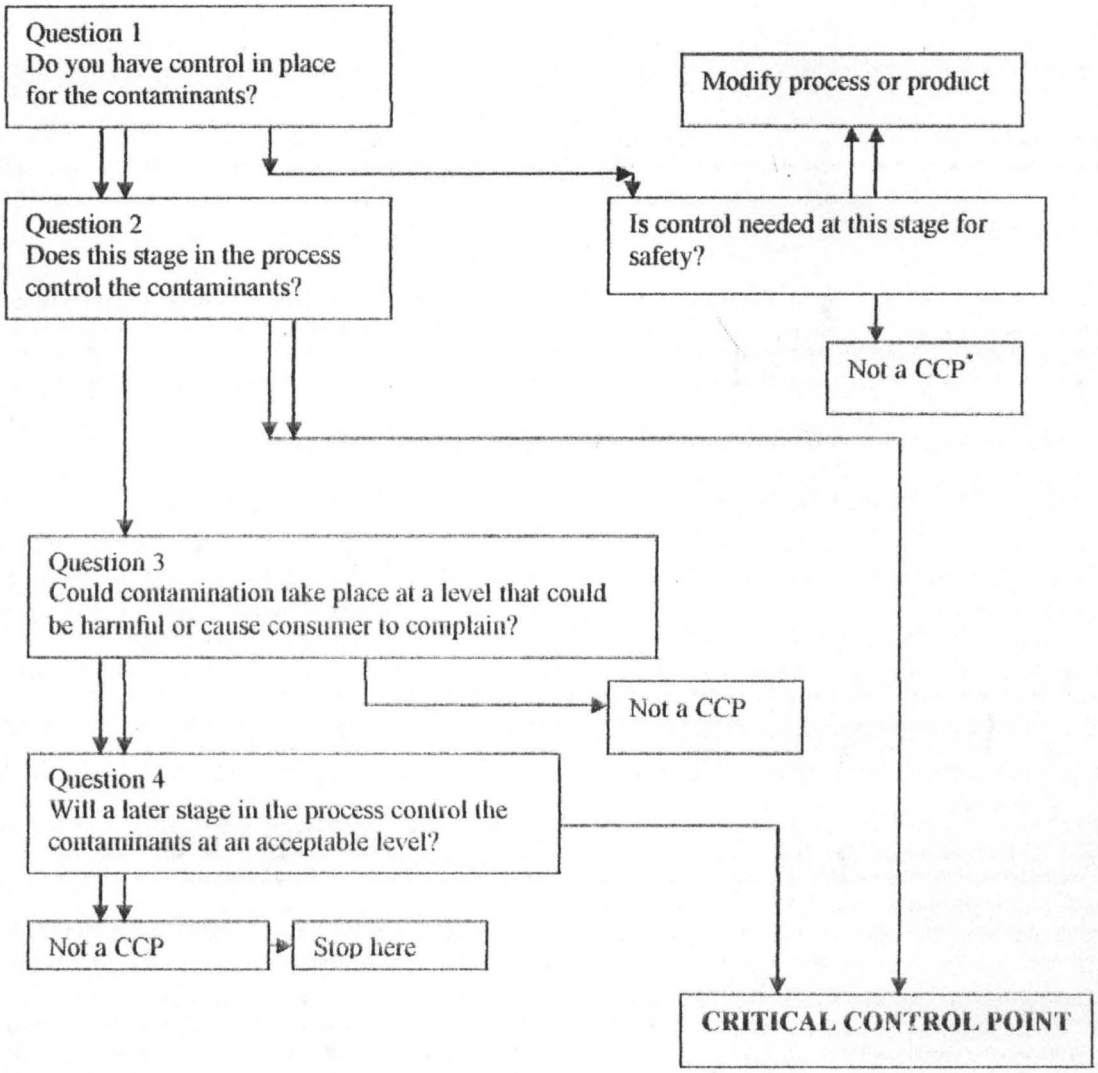
APPENDIX



Appendix 1 Critical control point decision tree

(Dilon and Griffith, 1995)

Appendix 2: Statistical analysis of data at 5% level of probability using analysis of variance (ANOVA).

Table of analysis

Source of variation	**Degree of freedom (d_f)**	**Sum of square (SS)**	**Mean sum of square (MSS)**	**F-Ratio**
				Cal. **Tab**
Between R	r-1	$\Sigma R^2/c - cf$	SSR/r-1	MSSR/MSE
Between C	c-1	$\Sigma C^2/r - cf$	SSC/c-1	MSSC/MSE
Error	rc-c-r+1	$S - \Sigma C^2/r - \Sigma R^2/c + cf$	SSE/Edf	
Total	rc-1	$S - cf$		

Correction factor (C.F) = $1/rcT^2$

R = row data

C = column data

r = number of row

c = number of column

T^2 = Grand total square

S = Sum of individual square

d_f = degree of freedom

1. Analysis of pH Results

Source of variation	df	SS	MSS	F_{cal}	F_{tab}
Between row	4	39.204	6.534	119.85	3**
Between column	6	0.3200	0.081	1.470	3*
Error	24	1.3084	0.055		
Total	34	40.832	1.201		

CV = 4.9%

- Not significantly different at 5% level of probability

** significant at 5% level of probability

2. Analysis of data from total soluble solids

Sources of variation	df	SS	MSS	F_{cal}	F_{tab}
Between row	6	1.615	0.269	198.36	3**
Between column	4	0.113	0.028	20.88	3**
Error	24	0.326	0.0014		
Total	34	1.761	0.052		

CV = 7.5%, ** significant at 5% level of probability

3. Analysis of Total Titratable Acidity data

Source of variation	df	SS	MSS	F_{cal}	F_{tab}
Between Row	4	163.72	27.287	24.82	3**
Between column	6	42.28	10.571	9.62	3**
Error	24	26.28	1.079		
Total	34	232.39	1.099		

CV = 4.6%, **Significant differences were observed among the processors in terms of their total acidity at 5% level of probability.

4. Change in viable counts in electrolyzed water

Sources of variation	df	SS	MSS	F_{cal}	F_{tab}
Between Row	8	50711.1	6338.89	0.450	0.859**
Between Column	1	6050	6050	0.430	0.535**
Error	8	112000	14000		
Total	17	168761	9927.12		

CV = 8.12, **There was significant difference observed in terms of bacteria counts in the in the two treatments at 5% level of probability.

5. Change in available chlorine concentration in soak water

Sources of variation	df	SS	MSS	F_{cal}	F_{tab}
Between row	8	8685.44	1085.68	5.12	0.017**
Between column	1	12.50	12.50	0.06	0.809*
Error	8	1695.00	211.88		
Total	17	10392.9	611.350		

CV = 7.9%, **Significant difference at 5% level of probability was observed among the results of available chlorine concentration from all the manufacturers.

6. Analysis of data for change in bacteria count in soak waters

Sources of variation	df	SS	MSS	F_{cal}	F_{tab}
Between Row	8	694257	86782.1	4.01	0.034**
Between Column	1	22.222	22.222	0.00	0.974*
Error	8	173103	21637.9		
Total	17	867382	51022.5		

*No significant difference was observed at 5% level of probability.

Appendix 3 Characterization of Bacteria Isolated from Raw Materials used in Soy- *kununzaki* Preparation

Raw materials	Gram reaction	Motility test	Glucose utilization	Catalase test	Indole test	Hydrolysis of starch	Organisms
Soybeans	+ve	-ve	GA	+ve	-ve	-ve	***Staphylococcus spp***
Soybeans	+ve	-ve	GA	+ve	+ve	+ve	***Bacillus spp***
Millet	-ve	-ve	G	+ve	+ve	-ve	***Shegilla spp***
Millet	+ve	-ve	GA	-ve	-ve	+ve	***Lactobacillus spp***
Ginger	-ve	-ve	GA	+ve	+ve	-ve	***Klebsiela spp***
Ginger	+ve	-ve	GA	+ve	+ve	+ve	***Bacillus spp***
Ginger	+ve	+ve	A	+ve	-ve	-ve	***Staphylococcus spp***
Black pepper	+ve	-ve	GA	+ve	-ve	+ve	***Bacillus spp***
Black pepper	+ve	-ve	GA	+ve	+ve	+ve	***Bacillus spp***
Cinnamon	-ve	-ve	GA	+ve	+ve	-ve	***Klebsiela spp***
Cloves	+ve	-ve	G	+ve	-ve	+ve	***Micrococcus spp***
Cloves	-ve	+ve	A	+ve	-ve	-ve	***Staphylococcus spp***

+ve = Positive result, -ve = Negative result, GA = Gas and Acid produced, G = Gas produced only, A = Acid produced only

Appendix 4 Morphological Characteristics and Identification of Fungi Isolated from raw materials used for Soy-*kununzaki* Preparation

Raw materials	Color of colony	Structure of hyphae	Shape and kind of asexual spores	Presence of special structure	Appearance of sporangiophore	Characteristics of spore head	Organisms
Millet	Black	Septate	Conidia in chain	Foot cell present	Long, erect, non-septate conidiophore	Numerous sterigmata	*Aspergillus niger*
Soybeans	Green	Septate	Conidia in chain	Foot cell present	Long, erect, non-septate conidiophore	Numerous strigmata	*Aspergillus niger*
Millet	White and cotton-like growth	Thick, non-septate	Round, black sporangiophore	Round collumela present	Unbranched sporongiophore	Round and black	*Mucor spp*
Clove	White and cotton-like growth	Septate	Conidia singly	Foot cell present	Long, erect septate conidiophore	Multi septate smoth walled conidia	*Trichophyton spp*
Ginger	Powdery and velvety	Septate	Conidia in chain	Foot cell absent	Rise vertically from hyphae	Broom like sterigmata	*Penicillium spp*
Cinnamon	Thick black colonies	Non-septate	Visible sporongia	Rhizoid present	Collapsed collumella	Broom-like sterigmata	*Rhizopus spp*

Appendix 5 Biochemical Characteristics and Identification of Yeast isolated from Soy-*kununzaki* During Production

Isolates	Fermentation					Assimilation					Urease test	Organisms*
	De	Ga	Su	Ma	La	De	Ga	Su	Ma	La		
213	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	***Trichosporon spp***
223	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	*Torulopsis spp*
322	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	*Sacharomyces cerevisiae*
112	+ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	*Candida spp*
344	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	*Candida spp*
342	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	*Cryptococcus neoformance*
442	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	*Candida spp*
142	+ve	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	
111	-ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	*Candida spp*
												Torulopsis spp

* Yeasts of the same genus are of different species. Dextrose (De), Galactose (Ga), Sucrose (Su), Maltose (Ma), Lactose (La).